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April 14, 2004

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APPLICATION NUMBER: *60/478,967*
FILING DATE: *June 16, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/00273*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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06-18-03

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17224 U.S. PTO 06/16/03

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PTO/SB/16 (05-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EV 319075848 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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Ehud	Arbit	Tarrytown, NY

☐ *Additional inventors are being named on the ________ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

NIGHT-TIME ORAL INSULIN THERAPY

CORRESPONDENCE ADDRESS

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☒ Customer Number 23280 → 23280 PATENT TRADEMARK OFFICE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 56 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 3 ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

☐ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0552

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $160

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ________

Respectfully submitted,

SIGNATURE [signature]

Date June 16, 2003

TYPED or PRINTED NAME Morey B. Wildes

REGISTRATION NO. (*if appropriate*) 36,968

TELEPHONE (212) 736-1940

Docket Number: 817.1013p3

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

S0479967 .051603

PTO/SB/17 (05-03)

Approved for use through 04/30/2003. OMB 0651-0032

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☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 160

Complete if Known	
Application Number	To Be Assigned
Filing Date	Herewith
First Named Inventor	GOLDBERG, et al.
Examiner Name	To Be Assigned
Group / Art Unit	To Be Assigned
Attorney Docket No.	817.1013p3

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	160

SUBTOTAL (1) ($) 160

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		- ** =	0	X		=	0
Independent Claims		- ** =	0	X		=	0
Multiple Dependent						=	0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	2451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,300	2453	650	Petition to revive – unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17 (q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) ____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY

Complete (*if applicable*)

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Signature	Morey B. Wildes			Date	June 16, 2003

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Re.: Docket No.: 817.1013p3
Applicant(s): GOLDBERG, et al.
Serial No.: To Be Assigned
Invention: NIGHT-TIME ORAL INSULIN THERAPY
Filing Date: Herewith

- Provisional Application for Patent Cover Sheet (1 page);
- Fee Transmittal (1 page);
- Specification, claims and Abstract (56 pages);
- Three (3) Sheets of Drawings; and
- Check in the amount of $160.

NIGHT–TIME ORAL INSULIN THERAPY

FIELD OF THE INVENTION

[0001] This invention relates to the oral delivery of therapeutic proteins in a therapeutically effective amount to the bloodstream. This invention further relates to oral administration of proteins as active agents as part of a therapeutic regimen. This invention further relates to the oral administration of insulin in a therapeutically effective amount for the treatment of diabetes. This invention further relates to compositions of a delivery agent and insulin for oral administration that facilitates insulin transport in a therapeutically effective amount to the bloodstream for the treatment of diabetes. This invention further provides methods for the preparation of a composition comprising insulin for oral administration.

[0002] The present invention further relates to methods for reducing adverse effects on the vascular system that are associated with insulin therapy. More specifically, the present invention relates to methods that reduce the incidence of diseases associated with systemic hyperinsulinemia. The present invention is also directed to oral pharmaceutical dosage forms that are administrable on a chronic basis to diabetics, in part to achieve such results.

BACKGROUND OF THE INVENTION

[0003] Proteins, carbohydrates and other biological molecules ("biological macromolecules") are finding increasing use in many diverse areas of science and technology. For example, proteins are employed as active agents in the fields of pharmaceuticals, vaccines and veterinary products. Unfortunately, the use of biological macromolecules as active agents in pharmaceutical compositions is often severely limited by the presence of natural barriers of passage to the location where the active agent is required. Such barriers include the skin, lipid bi-layers, mucosal membranes, severe pH conditions and digestive enzymes.

[0004] Oral delivery of active agents is a particularly desirable route of administration, because of safety and convenience considerations and because oral delivery replicates the physiologic

mode of insulin delivery. In addition, oral delivery provides for more accurate dosing than multidose vials and can minimize or eliminate the discomfort that often attends repeated hypodermic injections.

[0005] There are many obstacles to successful oral delivery of biological macromolecules. For example, biological macromolecules are large and are amphipathic in nature. More importantly, the active conformation of many biological macromolecules may be sensitive to a variety of environmental factors, such as temperature, oxidizing agents, pH, freezing, shaking and shear stress. In planning oral delivery systems comprising biological macromolecules as an active agent for drug development, these complex structural and stability factors must be considered.

[0006] In addition, in general, for medical and therapeutic applications, where a biological macromolecule is being administered to a patient and is expected to perform its natural biological function, delivery vehicles must be able to release active molecules, at a rate that is consistent with the needs of the particular patient or the disease process.

[0007] One specific biological macromolecule, the hormone insulin, contributes to the normal regulation of blood glucose levels through its release by the pancreas, more specifically by the ß-cells of a major type of pancreatic tissue (the islets of Langerhans). Insulin secretion is a regulated process which, in normal subjects, provides stable concentrations of glucose in blood during both fasting and feeding. Diabetes is a disease state in which the pancreas does not release insulin at levels capable of controlling glucose levels. Diabetes is classified into two types. The first type is diabetes that is insulin dependent and usually appears in young people. The islet cells of the pancreas stop producing insulin mainly due to autoimmune destruction and the patient must inject himself with the missing hormone. These Type 1 diabetic patients are the minority of total diabetic patients (up to 10% of the entire diabetic population). The second type of diabetes (type 2) is non-insulin dependent diabetes, which is caused by a combination of insulin resistance and insufficient insulin secretion. This is the most common type of diabetes in the Western world. Close to 8% of the adult population of various countries around the world, including the United States, have Type 2 diabetes, and about 30% of these patients will need to use insulin at some point during their life span due to secondary pancreas exhaustion.

[0008] Diabetes is the sixth leading cause of death in the United States and accounted for more than 193,000 deaths in 1997. However, this is an underestimate because diabetes contributes to substantially many deaths that are ultimately ascribed to other causes, such as cardiovascular disease. Complications resulting from diabetes are a major cause of morbidity in the population. For example, diabetic retinopathy is the leading cause of blindness in adults aged 20 through 74 years, and diabetic kidney disease accounts for 40% of all new cases of end-stage renal disease. Diabetes is the leading cause for amputation of limbs in the United States. Heart disease and strokes occur two to four times more frequently in adults with diabetes than in adult non-diabetics. Diabetes causes special problems during pregnancy, and the rate of congenital malformations can be five times higher in the children of women with diabetes.

[0009] The main cause of mortality with Diabetes Mellitus is long term micro- and macro-vascular disease. Cardiovascular disease is responsible for up to 80% of the deaths of Type II diabetic patients. See, for example, Kirpichnikov *et al.*, *Trends Endocrinol Metab 12*, 225-30 (2001); Garcia *et al.*, *Diabetes 23*, 105-11 (1974); Haffner *et al.*, *N Engl J Med 339*, 229-34 (1998); Sowers, *Arch Intern Med 158*, 617-21 (1998); Khaw, K. T. *et al.*, *Bmj 322*, 15-8 (2001). Diabetics have a two- to four-fold increase in the risk of coronary artery disease, equal that of patients who have survived a stroke or myocardial infarction. See, for example, Haffner *et al.*, *N Engl J Med 339*, 229-34 (1998); Sowers, *Arch Intern Med 158*, 617-21 (1998). This increased risk of coronary artery disease combined with an increase in hypertensive cardiomyopathy manifests itself in an increase in the risk of congestive heart failure. Stratton *et al.*, *Bmj 321*, 405-12 (2000); Shindler, D. M. *et al.*, *Am J Cardiol 77*, 1017-20 (1996). These vascular complications lead to neuropathies, retinopathies and peripheral vascular disease. See Kirpichnikov *et al.*, *Trends Endocrinol Metab 12*, 225-30 (2001). There is a need for diabetes treatments that will decrease the prevalence of such vascular disease in diabetes patients.

[0010] The beneficial effects of tight glycemic control on the chronic complications of diabetes are widely accepted in clinical practice. However, only recently it has been firmly established that elevated blood glucose levels are a direct cause of long-term complications of diabetes. The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) both showed that control of blood glucose at levels as close to normal

as possible prevents and retards development of diabetic retinopathy, nephropathy, neuropathy, and microvascular disease. Drug therapy of diabetes type II has consisted of oral antidiabetic agents and insulin if and when the oral agents fail. Insulin therapy in type I diabetes is essential and is intended to replace the absent endogenous insulin with an exogenous insulin supply. Because insulin is a protein drug (MW approx. 6000 Da) that is not absorbed in the gastrointestinal tract, it ordinarily requires parenteral administration such as by subcutaneous injection.

[0011] The problem of providing bioavailable unmodified human insulin, in a useful form, to the ever increasing population of diabetics has occupied physicians and scientists for almost 100 years. Many attempts have been made to solve some of the problems of stability and biological delivery of this small protein. Most diabetic patients self-administer insulin by daily subcutaneous injections. However, the limitations of multiple daily injections, such as inconvenience, poor patient acceptability, compliance and the difficulty of matching postprandial insulin availability to postprandial requirements, are some of the better known shortcomings of insulin therapy.

[0012] Despite studies demonstrating the beneficial effects of tight glycemic control on chronic complications of diabetes, clinicians are not particularly keen on aggressive insulin therapy, particularly in the early stages of the disease, and this is widely accepted in clinical practice. The unmet challenge of achieving tight glycemic control is due, in part, to the shortcomings of the available subcutaneous route of insulin administration and the fear of hypoglycemia. In addition to the practical limitations of multiple daily injections discussed above, the shortcomings of the commonly available subcutaneous route of insulin administration have resulted in the generally inadequate glycemic control associated with many of the chronic complications associated with diabetes. Elevated systemic levels of insulin lead to increased glucose uptake, glycogen synthesis, glycolysis, fatty acid synthesis and triacylglycerol synthesis, leading to the expression of key genes that result in greater utilization of glucose.

[0013] In the field of insulin delivery, where multiple repeated administrations are required on a daily basis throughout the patient's life, it would be desirable to create compositions of insulin that maintain protein tertiary structure so as not to alter physiological clinical activity and

stability and do not require injections. It would also be desirable to provide compositions of insulin that could be orally administrable, e.g., absorbed from the gastrointestinal tract in adequate concentrations, such that insulin is bioavailable and bioactive after oral administration. Oral absorption allows delivery directly to the portal circulation.

[0014] A method of providing insulin without the need for injections has been a goal in drug delivery. Insulin absorption in the gastrointestinal tract is prevented by its large size and enzymatic degradation. It would be desirable to create an oral pharmaceutical formulation of a drug such as insulin (which is not normally orally administrable due to, e.g., insufficient absorption from the gastrointestintal tract), which formulation would provide sufficient absorption and pharmacokinetic/pharmacodynamic properties to provide the desired therapeutic effect.

[0015] Insulin exemplifies the problems confronted in the art in designing an effective oral drug delivery system for biological macromolecules. The medicinal properties of insulin can be readily altered using any number of techniques, but its physicochemical properties and susceptibility to enzymatic digestion have precluded the design of a commercially viable oral or alternate delivery system.

SUMMARY OF THE INVENTION

[0016] It is one object of the present invention to provide useful oral pharmaceutical formulations of drugs that are not considered orally administrable due, e.g., to insufficient absorption of the drugs from the gastrointestinal tract, which formulations are therapeutically effective.

[0017] It is a further object of the present invention to provide useful pharmaceutical formulations of insulin for oral administration which are therapeutically effective.

[0018] It is a further object of the present invention to provide delivery agents that may be orally administered together with a drug that is not considered orally administrable due to, e.g., insufficient absorption of the drug from the gastrointestinal tract, so that the drug is absorbed in

adequate amounts from the gastrointestinal tract to provide the desired therapeutic effect, such as insulin.

[0019] It is an object of the present invention to provide compositions comprising a delivery agent and insulin for oral administration.

[0020] It is an object of the present invention to provide compositions of a delivery agent and insulin for oral administration that facilitates insulin transport in a therapeutically effective amount to the bloodstream for the treatment of diabetes, for the treatment of impaired glucose tolerance, for the purpose of achieving glucose homeostasis, for the treatment of early stage diabetes, for the treatment of late stage diabetes, and/or to serve as replacement for type I diabetic patients.

[0021] It is an object of the present invention to provide methods for the preparation of a composition comprising insulin and delivery agent for oral administration, which result in an orally administrable unit dose that provides a desired therapeutic effect.

[0022] It is an object of the present invention to provide a delivery agent(s) that can be utilized in an amount that facilitates the preparation of an oral unit dosage form of a drug that is not considered orally administrable by itself due to poor absorption, etc., and results in an orally administrable unit dose that provides a desired therapeutic effect.

[0023] It is a further object of the invention to provide a method and a pharmaceutical formulation which can reduce systemic blood insulin concentrations while providing therapeutically effective treatment of diabetes.

[0024] It is a further object of the invention to provide a method for prophylactically sparing beta cell function in a mammal which has impaired glucose tolerance or early stage diabetes.

[0025] It is a further object of the invention to provide a method for preventing beta cell death or dysfunction in a mammal which has impaired glucose tolerance or early stage diabetes.

[0026] It is a further object of the invention to provide a method for long term protection of a mammal which has impaired glucose tolerance or early stage diabetes mellitus from developing overt diabetes.

[0027] It is a further object of the invention to provide a method for delaying the onset of overt diabetes in a mammal which has impaired glucose tolerance or early stage diabetes mellitus.

[0028] In accordance with the above objects and others, the invention is directed in part to an oral solid dosage form comprising a dose of unmodified insulin that achieves a reduction in blood glucose concentration in human diabetic patients comparable to a subcutaneous insulin injection in those patients, while providing a lower (e.g., 20% or greater) totals dose of insulin in the peripheral blood circulation under acute, sub-acute and chronic conditions as compared to the peripheral blood insulin concentration obtained via the subcutaneous injection.

[0029] The invention is also directed in part to an oral solid dosage form comprising a dose of unmodified insulin that achieves a therapeutically effective reduction in blood glucose after oral administration to a human diabetic patient, and which maintains a physiological (portal/ peripheral) gradient, and in certain embodiments provides a ratio of portal vein insulin concentration to peripheral blood insulin concentration from about 2.5:1 to about 6:1, and preferably from about 4:1 to about 5:1.

[0030] The invention is further directed in part to an oral dosage form comprising a dose of unmodified insulin that achieves a therapeutically effective reduction in blood glucose after oral administration to human diabetic patients, the oral solid dosage form providing an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after oral administration to said patients, at least about 80% of the blood glucose concentration reduction caused by said dose of insulin occurring within about 2 hours after oral administration of said dosage form.

[0031] The invention is further directed in part to an oral dosage form comprising a therapeutically effective amount of unmodified insulin, said dosage form upon pre-prandial oral administration to human diabetic patients causing the post prandial mean plasma glucose concentration in said patients to be reduced for the first hour after oral administration relative to a

mean baseline (fasted) plasma glucose concentration (in the absence of sufficient insulin) in said patients.

[0032] The invention is further directed in part to an oral dosage form comprising a therapeutically effective amount of unmodified insulin, said oral dosage form upon pre-prandial oral administration provides a mean plasma glucose concentration which does not vary by more than about 40% (and more preferably not more than 30%) for the first hour after oral administration, relative to a mean baseline (fasted) plasma glucose concentration in said patients, where a meal is eaten by said patients within about one half hour of oral administration of said dosage form.

[0033] In preferred embodiments of the oral dosage forms of the invention described above, the oral dosage form is solid, and is preferably provided incorporated within a gelatin capsule or is contained in a tablet.

[0034] In certain preferred embodiments, the dose of unmodified insulin contained in the dosage form is from about 50 Units to about 600 Units (from about 2 to about 23mg), preferably from about 100 Units (3.8 mg) to about 450 Units (15.3 mg) insulin, and most preferably from about 150 Units (5.75 mg) to about 300 Units (11.5 mg), based on the accepted conversion of factor of 26.11 Units per mg.

[0035] In certain preferred embodiments, the dosage forms of the invention provide a t_{max} for insulin at about 0.1 to about 1.5 hours, and more preferably by about 0.25 to about 0.5 hours, after oral administration. In certain preferred embodiments, the t_{max} for insulin occurs at less than about 100 minutes after oral administration of the composition, preferably at less than about 45 minutes, more preferably at less than about 40 minutes, and still more preferably at about 22 minutes after oral administration of the composition. In certain preferred embodiments, the composition provides a t_{max} for glucose reduction at about 0.25 to about 1.5 hours, more preferably by about 0.75 to about 1.0 hours, after oral administration. In certain preferred embodiments, the t_{max} for glucose reduction occurs preferably at less than about 120 minutes, more preferably at less than about 80 minutes, and most preferably at about 45 minutes, after oral administration of the composition.

[0036] In certain preferred embodiments of the invention, the dosage forms begin delivering insulin into the portal circulation (via absorption through the mucosa of the stomach) to achieve peak levels within about 30 minutes or less.

[0037] In certain embodiments of the dosage forms described above, in the absence of a delivery agent, the dose of unmodified insulin is not adequately absorbed from the gastrointestinal tract when administered orally to render a desired effect. In certain preferred embodiments, in the absence of a delivery agent, the dose of insulin is not sufficiently absorbed when orally administered to a human patient to provide a desirable therapeutic effect but said dose provides a desirable therapeutic effect when administered to said patient by another route of adminstration. The invention in such embodiments is further directed to an oral dosage form comprising a dose of unmodified insulin together with a pharmaceutically acceptable delivery agent in an amount effective to facilitate the absorption of said insulin, such that a therapeutically effective amount of said dose of insulin is absorbed from the gastrointestinal tract of human diabetic patients.

[0038] In certain preferred embodiments, the pharmaceutical composition comprises from about 1 mg to about 800 mg of said delivery agent, preferably about 50 to about 600, more preferably from about 100 to about 400, most preferably about 200. In certain embodiments, the composition provides a peak plasma delivery agent concentration C_{max} from about 1,000 and about 150,000 ng/ml, and a t_{max} at about 0.25 to about 1.5 hours, and more preferably by about 0.25 to about 0.75 hours, most preferably 0.5 hours, after oral administration.

[0039] For purposes of the present invention, a preferred delivery agent is identified via chemical nomenclature as 4-[(4-chloro, 2-hydroxybenzoyl)amino]butanoic acid. In certain preferred embodiments, the delivery agent is a sodium salt, preferably monosodium salt. Alternatively, the same compound is identified by the alternative nomenclature monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate, or by the short name "4-CNAB".

[0040] The invention is further directed in part to a method of treatment of diabetes in humans, comprising administering one or more unit doses of the dosage forms described above and in further sections of the present specification.

[0041] The invention is further directed in part to a method of treatment of impaired glucose tolerance, achieving glucose homeostasis, early stage diabetes, and late stage diabetes in humans, comprising administering one or more unit doses of the dosage forms described above and in further sections of the present specification on a chronic basis.

[0042] The invention is also related to a method of orally treating mammals with an active agent (i.e., insulin) that is not sufficiently absorbed when orally administered to provide a desirable therapeutic effect but that provides a desirable therapeutic effect when administered by another route of adminstration, comprising orally administering said active agent together with a delivery agent which facilitates the absorption of insulin from the gastrointestinal tract, having one or more of the further characteristics set forth above.

[0043] The invention is further directed to a method of providing a therapeutically effective orally administrable unit dose of unmodified insulin, comprising combining from about 2 to about 23 mg of unmodified insulin with from about 100 to about 600 mg of a pharmaceutically acceptable delivery agent which facilitates absorption of said insulin from the gastrointestinal tract of human diabetic patients, and orally administering said unit dose to a human diabetic patient to provide a therapeutic effect. In preferred embodiments, the total weight of the unit dose is from about 102 mg to about 800 mg.

[0044] The present invention is also directed in part to a method of treating human diabetic patients comprising orally administering to human diabetic patients on a chronic basis an oral insulin treatment comprising a dose of unmodified insulin together with a delivery agent that facilitates the absorption of the dose of insulin from the gastrointestinal tract to provide a therapeutically effective reduction in blood glucose and a blood plasma insulin concentration that is reduced relative to the systemic blood insulin concentration of an equivalent therapeutically effective reduction in blood glucose concentration achieved by subcutaneous injection of insulin.

[0045] The invention is further directed to a method of treating diabetes and reducing the incidence of systemic hyperinsulinemia associated with chronic dosing of insulin, comprising orally administering on a chronic basis to a diabetic patient a dose of insulin and a delivery agent that facilitates the absorption of the dose of insulin from the gastrointestinal tract to provide a

therapeutically effective reduction and/or control in blood glucose and a mean systemic blood insulin concentration of the diabetic patient that is reduced relative to the mean systemic blood insulin concentration provided by subcutaneous injection of insulin in an amount effective to achieve equivalent reduction and/or control in a population of human diabetic patients.

[0046] The present invention is further directed to method for prophylactically sparing beta cell function in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin (as described herein) at nighttime. Preferably, the oral insulin formulation is administered to such human patients on a chronic basis, e.g., for at least about 2 weeks.

[0047] The present invention is further directed to a method for preventing beta cell death or dysfunction in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime. Preferably, the oral insulin formulation is administered to such human patients on a chronic basis at bedtime, e.g., for at least about 2 weeks.

[0048] The present invention is further directed to a method for long-term protection of a mammal which has impaired glucose tolerance or early stage diabetes mellitus from developing overt diabetes, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime. Preferably, the oral insulin formulation is administered to such human patients on a chronic basis at bedtime, e.g., for at least about 2 weeks.

[0049] The present invention is further directed to a method for delaying the onset of overt diabetes in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime. Preferably, the oral insulin formulation is administered to such human patients on a chronic basis at bedtime, e.g., for at least about 2 weeks.

[0050] The mean values of insulin concentration determination obtained in patients who have been administered subcutaneous insulin are well known to those skilled in the art.

[0051] The following terms will be used throughout the application as defined below:

[0052] Diabetic patient -- refers to humans suffering from a form of diabetes.

[0053] IGT -- means impaired glucose tolerance.

[0054] Diabetes -- is deemed to encompasses type 1 and type 2 diabetes, unless specifically specified otherwise.

[0055] Biological macromolecule -- biological polymers such as proteins and polypeptides. For the purposes of this application, biological macromolecules are also referred to as macromolecules.

[0056] Delivery agent -- refers to carrier compounds or carrier molecules that are useful in the oral delivery of therapeutic agents. "Delivery agent" may be used interchangeably with "carrier".

[0057] Therapeutically effective amount of insulin -- an amount of insulin included in the oral dosage forms of the invention which are sufficient to achieve a clinically significant control of blood glucose concentrations in a human diabetic patient either in the fasting state or in the fed state effective, during the dosing interval.

[0058] Effective amount of delivery agent -- an amount of the delivery agent that promotes the absorption of a therapeutically effective amount of the drug from the gastrointestinal tract.

[0059] Organic solvents -- any solvent of non-aqueous origin, including liquid polymers and mixtures thereof. Organic solvents suitable for the present invention include: acetone, methyl alcohol, methyl isobutyl ketone, chloroform, 1-propanol, isopropanol, 2-propanol, acetonitrile, 1-butanol, 2-butanol, ethyl alcohol, cyclohexane, dioxane, ethyl acetate, dimethylformamide, dichloroethane, hexane, isooctane, methylene chloride, tert-butyl alchohol, toluene, carbon tetrachloride, or combinations thereof.

[0060] Peptide -- a polypeptide of small to intermediate molecular weight, usually 2 or more amino acid residues and frequently but not necessarily representing a fragment of a larger protein.

[0061] Protein -- a complex high polymer containing carbon, hydrogen, oxygen, nitrogen and usually sulfur and composed of chains of amino acids connected by peptide linkages. Proteins in this application refer to glycoproteins, antibodies, non-enzyme proteins, enzymes, hormones and peptides. The molecular weight range for proteins includes peptides of 1000 Daltons to glycoproteins of 600 to 1000 kiloDaltons.

[0062] Reconstitution -- dissolution of compositions or compositions in an appropriate buffer or pharmaceutical composition.

[0063] Unit-Dose Forms-- refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. It is contemplated for purposes of the present invention that dosage forms of the present invention comprising therapeutically effective amounts of insulin may include one or more unit doses (e.g., tablets, capsules) to achieve the therapeutic effect.

[0064] Unmodified insulin – means insulin prepared in any pharmaceutically acceptable manner or from any pharmaceutically acceptable source which is not conjugated with an oligomer such as that described in U.S. Patent No. 6,309,633 and/or which not has been subjected to amphiphilic modification such as that described in U.S. Patent Nos. 5,359,030; 5,438,040; and/or 5,681,811.

[0065] As used herein, the phrase "equivalent therapeutically effective reduction" means that a maximal reduction of blood glucose concentration achieved by a first method of insulin administration (e.g. via oral administration of insulin in a patient(s)) is not more 20%, and preferably not more than 10% and even more preferably not more than 5% different from a maximal reduction of blood glucose concentration after administration by a second method (e.g., subcutaneous injection) in the same patient(s) or a different patient requiring the same reduction in blood glucose level.

[0066] The term "AUC" as used herein, means area under the plasma concentration-time curve, as calculated by the trapezoidal rule over the complete dosing interval, e.g., 24-hour interval.

[0067] The term "C_{max}" as it is used herein is the highest plasma concentration of the drug attained within the dosing interval.

[0068] The term "t_{max}" as it is used herein is the time period which elapses after administration of the dosage form at which the plasma concentration of the drug attains the C_{max} within the dosing interval.

[0069] The term "multiple dose" means that the human patient has received at least two doses of the drug composition in accordance with the dosing interval for that composition.

[0070] The term "single dose" means that the human patient has received a single dose of the drug composition and the drug plasma concentration has not achieved steady state.

[0071] Unless specifically designated as "single dose" or at "steady-state" the pharmacokinetic parameters disclosed and claimed herein encompass both single dose and steady-state conditions.

[0072] The term "mean", when preceding a pharmacokinetic value (e.g., mean t_{max}) represents the arithmetic mean value of the pharmacokinetic value unless otherwise specified.

[0073] The term "mean baseline level" means the measurement, calculation or level of a certain value that is used as a basis for comparison, which is the mean value over a statistically significant number of subjects, e.g., across a single clinical study or a combination of more than one clinical study.

[0074] The term "Bioavailability" as used herein means the degree or ratio (%) to which a drug or agent is absorbed or otherwise available to the treatment site in the body. This is calculated by the formula

$$\text{Rel. Bioavailability (\%)} = \frac{\text{Dose SC}}{\text{Dose Oral}} \times \frac{AUC_{INS}\ \text{Oral}}{AUC_{INS}\ \text{SC}} \times 100$$

[0075] The term "Biopotency" as used herein means the degree or ratio (%) to which a drug or agent is effective to the treatment site in the body. This is calculated by the formula

$$\text{Rel. Biopotency (\%)} = \frac{\text{Dose SC}}{\text{Dose Oral}} \times \frac{\text{AUC}_{\text{GIR}}\ \text{Oral}}{\text{AUC}_{\text{GIR}}\ \text{SC}} \times 100$$

[0076] As used herein and in the appended claims, the singular forms "a," "an," and "the," include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a molecule" includes one or more of such molecules, "a reagent" includes one or more of such different reagents, reference to "an antibody" includes one or more of such different antibodies, and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[0077] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods, compositions, reagents, cells, similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are described herein. All publications mentioned herein are incorporated herein, including all figures, graphs, equations, illustrations, and drawings, to describe and disclose specific information for which the reference was cited in connection with.

[0078] The publications discussed above are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than as limitations on the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0079] Figure 1 is a table of data (blood glucose, insulin and C-peptide) collected in the morning after nighttime dosing of insulin and 4-CNAB for each subject compared to that subject's own baseline levels.

[0080] Figure 2 is a bar graph showing the effect of nighttime dosing of insulin and 4-CNAB on blood glucose concentration.

[0081] Figure 3 is a bar graph showing the effect of nighttime dosing of insulin and 4-CNAB on blood C-peptide concentration.

[0082] Figure 4 is a bar graph showing the effect of nighttime dosing of insulin and 4-CNAB on blood insulin concentration.

DETAILED DESCRIPTION

[0083] Hyperinsulinemia (elevated blood concentrations of insulin) is caused by the administration of insulin in a location (and manner) which is not consistent with the normal physiological route of delivery. In normal healthy humans, insulin is released from the pancreas into the portal vein, which transfers the insulin to the liver. The liver utilizes a large portion of the insulin which it receives from the portal circulation. Glucose is the principal stimulus to insulin secretion in humans. Glucose enters the β cell by facilitated transport, and is then phosphorylated by glucokinase. Expression of glucokinase is primarily limited to cells and tissues involved in the regulation of glucose metabolism, such as the liver and the pancreatic β cells. The capacity of sugars to undergo phosphorylation and subsequent glycolysis correlates closely with their ability to stimulate insulin release. Insulin circulates in blood as the free monomer, and its volume distribution approximates the volume of extracellular fluid. Under fasting conditions, the concentration of insulin in portal blood is, e.g., about 2-4 ng/ml, whereas the systemic (peripheral) concentration of insulin is, e.g., about 0.5 ng/ml, in normal healthy humans, translating into, e.g., a 5:1 ratio.

[0084] Insulin is administered parenterally, usually by subcutaneous injection. In human diabetics who receive insulin via subcutaneous injection, the ratio is changed to about 0.75:1. Thus, in such diabetic patients, the liver does not receive the necessary concentrations of insulin to adequately control blood glucose.

[0085] It has been an unmet goal in the art to imitate normal insulin levels in the portal and systemic circulation via oral administration of insulin. By virtue of the present invention, the

ratio of portal (unmodified) insulin concentration to systemic (unmodified) insulin concentration approaches in human diabetic patients approaches that which is obtained in normal healthy humans. The chronic administration of oral dosage forms of the present invention result in a higher portal insulin concentration and lower systemic insulin concentration over time than that obtained with an equi-effective dose of insulin administered subcutaneously (i.e., which provide similar control of blood glucose levels). By virtue of the present invention, lower levels of hyperinsulinemia are obtained, e.g., systemic insulin concentrations are at least about 20% lower when compared to a comparably effective subcutaneous dose of insulin. Transient peaks in insulin levels which may occur by virtue of the oral administration of insulin in accordance with the present invention is not believed to be associated with vascular diseases.

[0086] Typically, insulin is not absorbed to any extent through the gastrointestinal tract, presumably due to its size and potential for enzymatic degradation. The present invention provides pharmaceutical compositions that are useful as delivery agents in the oral delivery of an active agent that is not generally considered by those skilled in the art to be administrable via the oral route, such as insulin. Such compositions serve to make insulin bioavailable and absorbable through the gastrointestinal mucosa when orally administered.

[0087] The present invention provides a method of administering insulin and pharmaceutical compositions useful for administering insulin such that the insulin is bioavailable and absorbable from the gastrointestinal tract and such that the incidence of vascular diseases normally associated with chronic dosing of insulin is attenuated. The delivery agents of the invention enable insulin to be orally absorbable through the mucosa of the stomach. Following oral administration of the pharmaceutical compositions of the present invention, the delivery agent passes though the mucosal barriers of the gastrointestinal tract and is absorbed into the blood stream where it can be detected in the plasma of subjects. The level of delivery agent in the bloodstream as measured in the plasma is dose-dependent. The delivery agent facilitates the absorption of insulin administered therewith (either in the same dosage form, or simultaneously therewith), or sequentially (in either order, as long as both the delivery agent and insulin are administered within a time period which provides both in the same location, e.g., the stomach, at the same time). As disclosed below, oral administration of insulin, in particular using the

delivery agents disclosed herein, effectively reduces the incidence of vascular and other disease states that are associated with traditional dosing of insulin, i.e., subcutaneously.

[0088] The preferred pharmaceutical compositions of the invention comprise a combination of insulin and a delivery agent in a suitable pharmaceutical carrier or excipient as understood by practitioners in the art. The means of delivery of the pharmaceutical composition can be, for example, a capsule, compressed tablet, pill, solution, freeze-dried, powder ready for reconstitution or suspension suitable for administration to the subject.

[0089] The pharmaceutical compositions and method of the present invention provide a number of advantages in addition to convenience, acceptance and patient compliance. Insulin absorbed in the gastrointestinal tract mimics the physiology of insulin secreted by the pancreas because both are released into the portal vein and carried directly to the liver. Absorption into the portal circulation maintains a peripheral-portal insulin gradient that regulates insulin secretion. The present invention comprises pharmaceutical compositions and method for oral insulin delivery that enable achieving low blood glucose without having high levels of systemic insulin.

[0090] Preferably, the pharmaceutical composition includes insulin as the active agent. As used herein, "insulin" refers to insulin from a variety of sources. Naturally occurring insulin and structurally similar bioactive equivalents (insulin analogues including short acting and analogues with protracted action) can be used. Insulin useful in the invention can be isolated from different species of mammal. For example, animal insulin preparations extracted from bovine or porcine pancreas can be used. Insulin analogues, derivatives and bioequivalents thereof can also be used with the invention. In addition to insulin isolated from natural sources, the present invention can use insulin chemically synthesizing using protein chemistry techniques such as peptide synthesis. Analogues of insulin are also suitable for the present invention.

[0091] The insulin used in the present invention may be obtained by isolating it from natural sources or by chemically synthesizing it using peptide synthesis, or by using the techniques of molecular biology to produce recombinant insulin in bacteria or eucaryotic cells. Analogs of insulin are also provided by the present invention. Insulin from other species of mammal may

also be used in the present invention. The physical form of insulin may include crystalline and/or amorphous solid forms. In addition, dissolved insulin may be used. Other suitable forms of insulin, including, but not limited to, synthetic forms of insulin, are described in U.S. Patents Nos. 4,421,685, 5,474,978, and 5,534,488, the disclosure of each of which is hereby incorporated by reference in its entirety.

[0092] The most preferred insulin useful in the pharmaceutical compositions and methods of the present invention is human recombinant insulin. Human recombinant insulin can be prepared using genetic engineering techniques that are well known in the art. Recombinant insulin can be produced in bacteria or eucaryotic cells. Functional equivalents of human recombinant insulin are also useful in the invention. Recombinant human insulin can be obtained from a variety of commercial sources. For example, insulin (Zinc, human recombinant) can be purchased from Calbiochem (San Diego, CA). Alternatively, human recombinant Zinc-Insulin Crystals: Proinsulin Derived (Recombinant DNA Origin) USP Quality can be obtained from Eli Lilly and Company (Indianapolis, IN). All such forms of insulin, including insulin analogues (including but not limited to Insulin Lispro, Insulin Aspart, Insulin Glargine, Insulin Detemir) are deemed for the purposes of this specification and the appended claims are considered to be encompassed by the term "insulin."

[0093] The present invention provides compositions of recombinant human zinc insulin and a delivery agent as a drug for oral administration of insulin in humans.

[0094] In yet further embodiments of the invention, the active agent is not insulin but instead is an active agent of a biological nature suitable for use in the present invention including, but not limited to, proteins; polypeptides; peptides; hormones; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; other organic compounds; and particularly compounds which by themselves do not pass (or which pass as only a fraction of the administered dose) through the gastro-intestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastro-intestinal tract; or any combination thereof. Further examples of active agents of a biological nature include, but are not limited to, the following, including synthetic, natural or recombinant sources thereof: growth hormones, including human growth hormones (hGH), recombinant human growth hormones (rhGH), bovine growth

hormones, and porcine growth hormones; growth hormone-releasing hormones; interferons, including α, β and γ; interleukin-1; interleukin-2; insulin, including porcine, bovine, human, and human recombinant, optionally having counter ions including sodium, zinc, calcium and ammonium; insulin-like growth factor, including IGF-1; heparin, including unfractionated heparin, heparinoids, dermatans, chondroitins, low molecular weight heparin, very low molecular weight heparin and ultra low molecular weight heparin; calcitonin, including salmon, eel, porcine and human; erythropoietin; atrial naturetic factor; antigens; monoclonal antibodies; somatostatin; protease inhibitors; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; leutinizing-hormone-releasing-hormone; follicle stimulating hormone; glucocerebrosidase; thrombopoietin; filgrastim; prostaglandins; cyclosporin; vasopressin; cromolyn sodium (sodium or disodium chromoglycate); vancomycin; desferrioxamine (DFO); parathyroid hormone (PTH), including its fragments; antimicrobials, including anti-fungal agents; vitamins; analogs, fragments, mimetics or polyethylene glycol (PEG)-modified derivatives of these compounds; or any combination thereof.

[0095] In one embodiment of this invention, the protein active agents have a molecular weight of less than or equal to 10,000 Daltons. In another embodiment of this invention, protein active agents have a molecular weight of about 6,000 Daltons. In another embodiment of this invention, protein active agents have a molecular weight of greater than or equal to 10,000 Daltons. According to an alternate embodiment of the present invention, protein active agents have a molecular weight that is greater than or equal to 20,000 Daltons. In a further embodiment, protein active agents have a molecular weight that is greater than or equal to 30,000 Daltons. According to an alternate embodiment, protein active agents have a molecular weight that is greater than or equal to 40,000 Daltons. According to another alternate embodiment, protein active agents have a molecular weight that is greater than or equal to 50,000 Daltons.

[0096] Insulin entry into the bloodstream produces a decrease in plasma glucose levels. Therefore, oral absorption of insulin may be verified by observing the effect on a subject's blood sugar following oral administration of the composition. In a preferred embodiment of the invention, the oral dosage forms of the invention facilitate the oral delivery of insulin, and after insulin is absorbed into the bloodstream, the composition produces a maximal decrease in blood

glucose in treated patients from about 20 to about 60 minutes after oral administration. In another embodiment of the present invention, the pharmaceutical composition produces a maximal decrease in blood glucose in treated patients from about 30 to about 50 minutes post oral administration. More particularly, the pharmaceutical composition produces a maximal decrease in blood glucose in treated patients at about 40 minutes after oral administration.

[0097] The magnitude of the decrease in blood glucose produced by insulin absorbed into the bloodstream following entry into the gastrointestinal tract varies with the dose of insulin. In certain embodiments of the invention, human diabetic patients show a maximal decrease in blood glucose by at least 10% within one hour post oral administration. In another embodiment, human diabetic patients show a maximal decrease in blood glucose by at least 20% within one hour post oral administration, alternatively, at least 30% within one hour post oral administration.

[0098] Normal levels of blood glucose vary somewhat throughout the day and in relation to the time since the last meal. One goal of the present invention is to provide oral compositions of insulin that facilitate achieving close to normal levels of blood glucose throughout the 24-hour daily cycle. In a preferred embodiment of the invention, wherein the pharmaceutical composition includes insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve a fasting blood glucose concentration from about 90 to about 110 mg/dl. In another preferred embodiment of the invention, wherein the pharmaceutical composition includes insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve a fasting blood glucose concentration from about 95 to about 105 mg/dl, more preferably, the subject manifests fasting blood glucose concentrations at about 100 mg/dl.

[0099] In the time after a meal is consumed, blood glucose concentration rises in response to digestion and absorption into the bloodstream of carbohydrates derived from the food eaten. The present invention provides oral compositions of insulin that prevent or control very high levels of blood glucose from being reached and/or sustained. More particularly, the present invention provides compositions which facilitate achieving normal levels of blood glucose after a meal has been consumed, i.e., post-prandial. In a preferred embodiment of the invention, the pharmaceutical composition includes insulin as the active agent and a delivery agent in an amount effective to achieve a post-prandial blood glucose concentration from about 130 to about

170 mg/dl. In another preferred embodiment of the invention, the pharmaceutical composition includes insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve a post-prandial blood glucose concentration from about 140 to about 160 mg/dl, more preferably, the subject manifests fasting blood glucose concentrations at less than about 160 mg/dl.

[00100] The present invention provides pharmaceutical compositions for oral administration which includes insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve pre-prandial (before a meal is consumed) blood glucose concentration from about 95 to about 125 mg/dl. In a preferred embodiment, the present invention provides pharmaceutical compositions for oral administration which includes insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve pre-prandial blood glucose concentration from about 100 to about 120 mg/dl.

[00101] The present invention provides pharmaceutical compositions for oral administration which include insulin as the active agent and a delivery agent in an amount effective to achieve blood glucose concentrations within the normal range during the evening period from about 70 to about 120 mg/dl. In a preferred embodiment, the present invention provides pharmaceutical compositions for oral administration which include insulin or an insulin analog as the active agent and a delivery agent in an amount effective to achieve blood glucose concentrations at 3 AM from about 80 to about 120 mg/dl.

[00102] In certain preferred embodiments, the methods and pharmaceutical compositions provide the pharmacokinetic parameters set forth in United States Provisional Applications Nos. 60/346,746 and 60/347,312, the disclosure of each of which is incorporated herein by reference.

[00103] The amount of delivery agent necessary to adequately deliver insulin into the blood stream of a subject needing the therapeutic effect of insulin can vary depending on one or more of the following; chemical structure of the particular delivery agent; the nature and extent of interaction of insulin and the delivery agent; the nature of the unit dose, i.e., solid, liquid, tablet, capsule, suspension; the concentration of delivery agent in the GI tract, the feeding state of the subject, the diet of the subject, the heath of the subject and the ratio of delivery agent to insulin.

[00104] In preferred embodiments, the oral dosage forms of the present invention comprise a mixture of insulin and a delivery agent, e.g., monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate (4-CNAB), a novel compound discovered by Emisphere Technologies, Inc., or separately containing insulin and the delivery agent.

[00105] In further embodiments of the present invention, the oral dosage forms described herein are orally administered as described herein in combination with an additional therapy to treat diabetes, impaired glucose tolerance, or to achieve glucose homeostasis, said additional therapy comprising, for example, an additional drug such as sulfonylurea, a biguanide, an alpha-glucosidase, insulin delivered via a different pathway (e.g., parenteral insulin), and/or an insulin sensitizer.

[00106] In further embodiments of the invention, the oral dosage forms described herein reduce the likelihood of hypoglycemic events, mainly because of two reasons: (a) one cannot hyperinsulinize the liver, because even under hyperinsulinemia the liver uptake of glucose will be unchanged. Unlike the peripheral tissue, the liver will only cease producing endogenous insulin and not sequester additional glucose; and (b) the short peak of insulin (e.g., as shown in the appended examples) shows that even if insulin were to reach high peripheral levels, the peak drops precipitously.

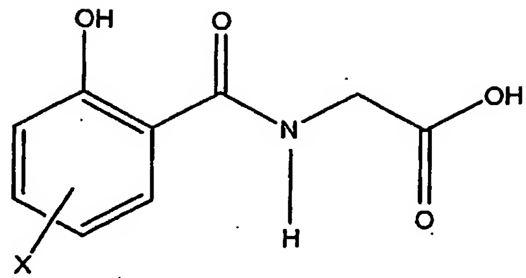
[00107] The effect of absorption of insulin is manifested in human patients treated with the pharmaceutical compositions of the present invention by observing reductions in C-peptide concentration following oral treatment. For example, in one embodiment of the invention, the pharmaceutical composition comprises insulin as the active agent and the compound 4-CNAB as a delivery agent to facilitate the oral delivery of insulin, and, after insulin is absorbed into the bloodstream, the composition produces a maximal decrease in C-peptide concentration in treated patients from about 80 and about 120 minutes post oral administration. More particularly, the composition produces a maximal decrease in C-peptide concentration in treated patients from about 90 and about 110 minutes post oral administration.

[00108] Absorption of insulin can be detected in subjects treated with the pharmaceutical compositions of the present invention by monitoring the plasma levels of insulin after treatment.

The time it takes for an active agent to reach a peak in the bloodstream (t_{max}) may depend on many factors such as the following: the nature of the unit dose, i.e., solid, liquid, tablet, capsule, suspension; the concentration of active agent and delivery agent in the GI tract; the feeding state of the subject; the diet of the subject; the health of the subject and the ratio of active agent to the delivery agent. In a preferred embodiment of the invention, wherein the pharmaceutical composition includes the compound 4-CNAB as the delivery agent and insulin as the active agent, the composition provides a peak plasma insulin concentration from about 0.1 to about 1 hour after oral administration. In another embodiment, the composition provides a peak plasma insulin concentration from about 0.2 to about 0.6 hours after oral administration. In a preferred embodiment, the composition provides a peak plasma insulin concentration from about 0.3 to about 0.4 hours after oral administration. In another embodiment, the composition provides a peak plasma insulin concentration within about 1 hour after oral administration. In certain preferred embodiments of the invention, the pharmaceutical composition comprises insulin as the active agent and the compound 4-CNAB as a delivery agent to facilitate the oral delivery of insulin, and after insulin is absorbed into the bloodstream, the plasma insulin levels in treated patients peak at about 20 minutes post oral administration with a second peak at about 105 minutes.

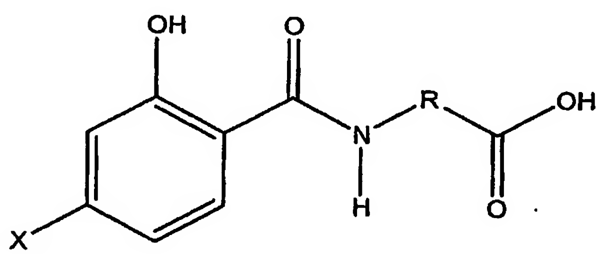
[00109] In preferred embodiments, the compositions of the present invention include an active agent (e.g., insulin) and a delivery agent that serves to render the active agent orally absorbable through the mucosa of the stomach. Accordingly, the present invention solves the problem of oral absorption of macromolecules by providing delivery agents that facilitate transport of such biomolecules through the gastrointestinal system and into the bloodstream where the active agent can perform its necessary biological role. As a result of the present invention, effective oral drug delivery methods are provided to increase the oral bioavailability and absorption of drugs that are currently administered parenterally.

[00110] In other preferred embodiments, the delivery agents used in the invention have the following structure:



wherein X is one or more of hydrogen, halogen, hydroxyl or C_1-C_3 alkoxy, and R is substituted or unsubstituted C_1-C_3 alkylene, substituted or unsubstituted C_1-C_3 alkenylene.

[00111] In certain preferred embodiments, the delivery agents of the invention preferably have the following structure:



wherein X is halogen, and R is substituted or unsubstituted C_1-C_3 alkylene, substituted or unsubstituted C_1-C_3 alkenylene.

[00112] In a preferred embodiment of the present invention, the pharmaceutical composition includes a delivery agent wherein X is chlorine and R is C_3 alkylene. In another preferred embodiment of the present invention, the pharmaceutical composition includes the compound 4-[(4-chloro, 2-hydroxybenzoyl)amino]butanoic acid as a delivery agent for the oral delivery of insulin, preferably the monosodium salt thereof.

[00113] The delivery agents may be in the form of the carboxylic acid or salts thereof. Suitable salts include, but are not limited to, organic and inorganic salts, for example alkali-metal salts, such as sodium, potassium and lithium; alkaline-earth metal salts, such as magnesium, calcium or barium; ammonium salts; basic amino acids, such as lysine or arginine; and organic amines,

such as dimethylamine or pyridine. Preferably, the salts are sodium salts. The salts may be mono- or multi-valent salts, such as monosodium salts and di-sodium salts. The salts may also be solvates, including ethanol solvates, and hydrates.

[00114] Other suitable delivery agents that can be used in the present invention include those delivery agents described United States Patents Nos. 5,650,386, 5,773,647, 5,776,888, 5,804,688, 5,866,536, 5,876,710, 5,879,681, 5,939,381, 5,955,503, 5,965,121,5,989,539, 5,990,166, 6,001,347, 6,051,561, 6,060,513, 6,090,958, 6,100,298, 5,766,633, 5,643,957, 5,863,944, 6,071,510 and 6,358,504, the disclosure of each of which is incorporated herein by reference. Additional suitable delivery agents are also described in International Publications Nos. WO 01/34114, WO 01/21073, WO 01/41985, WO 01/32130, WO 01/32596, WO 01/44199, WO 01/51454, WO 01/25704, WO 01/25679, WO 00/50386, WO 02/02509, WO 00/47188, WO 00/07979, WO 00/06534, WO 98/25589, WO 02/19969, WO 00/59863, WO 95/28838, WO 02/20466 and WO 02/19969, and International Patent Applications Nos. PCT/US02/06610 and PCT/US02/06295, the disclosure of each of which is incorporated herein by reference.

[00115] Salts of the delivery agent compounds of the present invention may be prepared by methods known in the art. For example, sodium salts may be prepared by dissolving the delivery agent compound in ethanol and adding aqueous sodium hydroxide.

[00116] The compounds described herein may be derived from amino acids and can be readily prepared from amino acids by methods known by those with skill in the art based upon the present disclosure and the methods described in International Publications Nos. WO 96/30036, WO 97/36480, WO 98/34632 and WO 00/07979, and in United States Patents Nos. 5,643,957 and 5,650,386, the disclosure of each of which is incorporated herein by reference. For example, the compounds may be prepared by reacting the single amino acid with the appropriate acylating or amine-modifying agent, which reacts with a free amino moiety present in the amino acid to form amides. Protecting groups may be used to avoid unwanted side reactions as would be known to those skilled in the art.

[00117] The delivery agents may also be prepared by the methods of International Patent Application No. PCT/US01/21073, the disclosure of which is incorporated herein by reference.

[00118] The delivery agents may also be prepared by alkylation of the appropriate salicylamide according to the methods of International Publication No. WO 00/46182, the disclosure of which is incorporated herein by reference. The salicylamide may be prepared from salicylic acid via the ester by reaction with sulfuric acid and ammonia.

[00119] In addition, poly amino acids and peptides comprising one or more of these compounds may be used. An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids. Poly amino acids are either peptides (which are two or more amino acids joined by a peptide bond) or are two or more amino acids linked by a bond formed by other groups which can be linked by, e.g., an ester or an anhydride linkage. Peptides can vary in length from dipeptides with two amino acids to polypeptides with several hundred amino acids.

[00120] The delivery agent compound may be purified by recrystallization or by fractionation on one or more solid chromatographic supports, alone or linked in tandem. Suitable recrystallization solvent systems include, but are not limited to, ethanol, water, heptane, ethyl acetate, acetonitrile, methanol and tetrahydrofuran and mixtures thereof. Fractionation may be performed on a suitable chromatographic support such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase chromatography using trifluoroacetic acid/ acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water or an appropriate buffer as the mobile phase. When anion exchange chromatography is performed, preferably a 0-500 mM sodium chloride gradient is employed.

[00121] Following oral administration of the pharmaceutical compositions of the present invention, the delivery agent passes though the mucosal barriers of the GI tract and is absorbed into the blood stream where it can be detected in the plasma of subjects. The level of delivery agent in the bloodstream as measured in the plasma is dose-dependent. The delivery agent facilitates the absorption of the drug (active agent) administered therewith (either in the same dosage form, or simultaneously therewith), or sequentially (in either order, as long as both the delivery agent and the drug are administered within a time period which provides both in the same location, e.g., the stomach, at the same time).

[00122] In certain preferred embodiments of the invention, a peak plasma concentration (C_{max}) of the delivery agent achieved after oral administration is preferably from about 10 to about 250,000 ng/ml, after oral administration, preferably from about 100 to about 125,000, and preferably the peak plasma concentration of the delivery agent is from about 1,000 to about 50,000 ng/ml, after oral administration. More preferably, the peak plasma concentration of the delivery agents of the present invention is from about 5,000 to about 15,000 ng/ml, after oral administration.

[00123] The time it takes for the delivery agent to reach a peak in the bloodstream (t_{max}) may depend on many factors such as the following: the nature of the unit dose, i.e., solid, liquid, tablet, capsule, suspension; the concentration of delivery agent in the GI tract; the feeding state of the subject; the diet of the subject; the health of the subject and the ratio of delivery agent to the active agent. The delivery agents of the present invention are rapidly absorbed from the gastrointestinal tract when orally administered in an immediate release dosage form, and preferably provide a peak plasma concentration within about 0.1 to about 8 hours after oral administration, and preferably at about 0.1 to about 3 hours after oral administration.

[00124] In preferred embodiments, the t_{max} of the delivery agent occurs at about 0.3 to about 1.5 hours after oral administration. In certain embodiments, the delivery agent achieves a t_{max} within about 2 hours after oral administration, and most preferably, within about 1 hour after oral administration.

[00125] The amount of delivery agent necessary to adequately deliver an active agent into the blood stream of a subject needing the therapeutic effect of that active agent may vary depending on one or more of the following; the chemical nature of the active agent; the chemical structure of the particular delivery agent; the nature and extent of interaction from about the active agent and delivery agent; the nature of the unit dose, i.e., solid, liquid, tablet, capsule, suspension; the concentration of delivery agent in the GI tract; the feeding state of the subject; the diet of the subject; the health of the subject and the ratio of delivery agent to the active agent. In a certain preferred embodiment of the invention, the amount of the delivery agent preferred for the pharmaceutical composition is from about 1 mg to about 2,000 mg delivery agent, more preferably from about 1 mg to about 800 mg of said delivery agent, more preferably from about

50 mg to about 700 mg of said delivery agent, even more preferably from about 70 mg to about 700 mg of said delivery agent, still more preferably from about 100 to about 600 mg.

[00126] Preferably, the delivery agent is 4-CNAB. Since the amount of delivery agent required to deliver a particular active agent is variable and the amount of active agent required to produce a desired therapeutic effect is also a variable, the ratio of active agent to delivery agent may vary for different active agent/delivery agent combinations. In certain preferred embodiments of the invention where the oral pharmaceutical composition includes insulin as the active agent and the delivery agent is the compound 4-CNAB, the amount of the delivery agent included in the pharmaceutical composition may be from about 100 mg to about 600 mg of said delivery agent.

[00127] In certain preferred embodiments of the invention, the pharmaceutical composition includes insulin as the active agent and the delivery agent is the monosodium salt of 4-CNAB, the ratio of insulin [Units] to delivery agent [mg] ranges from 10:1 [Units/mg] to 1:10 [Units/mg], preferably, the ratio of insulin [Units] to delivery agent [mg] ranges from 5:1 [Units/mg] to 0.5:1 [Units/mg].

[00128] Preferred insulin doses in a single administration are about 5 to about 1000 insulin units USP, preferably from about 50 to about 400, more preferably from about 150 to about 400, and still more preferably from about 150 to about 300 units.

[00129] The optimum ratio of insulin to delivery agent can vary depending on the delivery agent. Optimizing the ratio of insulin to delivery agent is within the knowledge of one skilled in the art.

[00130] In a preferred embodiment of the invention, wherein the pharmaceutical composition includes the compound 4-CNAB as the delivery agent and insulin as the active agent, the composition provides a peak plasma delivery agent concentration within about 0.1 to about 3 hours after oral administration. In certain preferred embodiments where the pharmaceutical composition includes the compound 4-CNAB as the delivery agent and insulin as the active agent, the peak plasma concentration of delivery agent attained is from about 8,000 to about 37,000 ng/ml.

[00131] The mechanism by which 4-CNAB facilitates the gastrointestinal absorption of insulin has not yet been fully elucidated. The current working hypothesis is that 4-CNAB interacts with insulin non-covalently, creating more favorable physicochemical properties for absorption. This working hypothesis is provided for explanation purposes only and is not intended to limit the present invention or the appended claims in any way.

[00132] The delivery agent may be used directly by mixing one or more such agents with the active agent (e.g., unmodified insulin) prior to administration. The delivery agent and active agent may be mixed in dry powder form or wet granulated together. To this mixture, other pharmaceutically acceptable excipients may be added. The mixture may be then tableted or placed into gelatin capsules containing a unit dose of the active agent and the delivery agent. Alternatively, the delivery agent/active agent mixture may be prepared as an oral solution or suspension. The delivery agent and active agent do not need to be mixed together prior to administration, such that, in certain embodiments, the unit dose of active agent (with or without other pharmaceutically acceptable excipients) is orally administered without the delivery agents of this invention, and the delivery agent is separately orally administered (with or without other pharmaceutically acceptable excipients) before, after, or simultaneously with the active agent.

[00133] In certain preferred embodiments, the oral dosage forms of the present invention are solid. The unmodified insulin in dry powder form is stable, and in certain preferred embodiments is simply mixed in a desirable ratio with the delivery agent. The dry powder mixture may then be filled into gelatin capsules, with or without optional pharmaceutical excipients. Alternatively, the unmodified insulin in dry powder form may be mixed with the delivery agent together with optional pharmaceutical excipients, and the mixture may be tableted in accordance with standard tableting procedures known to those having ordinary skill in the art.

[00134] The present invention also provides methods for treating human diabetic patients with active agents that are not inherently bioavailable, such as for example treating diabetics with insulin. More particularly, the present invention provides method of treating humans with an oral dosage form of a pharmaceutical composition, wherein the pharmaceutical composition includes the following: first, an active agent or a pharmaceutically acceptable salt thereof, which is not orally bioavailable when dissolved or suspended in aqueous solution, wherein the active

agent provide a therapeutic effect when administered to a subject by another means (e.g., via subcutaneous injection); and, second, an effective amount of a delivery agent or a pharmaceutically acceptable salt thereof, which renders the active agent orally absorbed (e.g., bioavailable). In certain embodiments, the method comprises the following steps: first, contacting the active agent (e.g., insulin) with said delivery agent, and thereafter orally administering the pharmaceutical composition. Alternatively, the method comprises administering the insulin and the delivery agent in such a manner that the insulin and delivery agent contact each other in-vivo (e.g., in the stomach), such that the delivery agent is available to facilitate absorption of the insulin through the stomach mucosa.

[00135] The dosage forms of the present invention may be produced by first dissolving the active agent and delivery agents into one solution or separate solutions. The solvent will preferably be an aqueous solution, but organic solvents or aqueous organic solvent mixtures may be used when necessary to solubilize the delivery agent. If two solutions are used, the proportions of each necessary to provide the correct amount of either active agent or delivery agent are combined and the resulting solution may be dried, by lyophilization or equivalent means. In one embodiment of the invention, the oral dosage form may be dried and rehydrated prior to oral administration.

[00136] The administration mixtures may be prepared, e.g., by mixing an aqueous solution of the delivery agent with an aqueous solution of the active ingredient, such as insulin, just prior to administration. Alternatively, the delivery agent and the biologically or chemically active ingredient can be admixed during the manufacturing process. The solutions may optionally contain additives such as phosphate buffer salts, citric acid, acetic acid, gelatin, and gum acacia.

[00137] Stabilizing additives may be incorporated into the delivery agent solution. With some drugs, the presence of such additives promotes the stability and dispersibility of the agent in solution. The stabilizing additives may be employed at a concentration ranging from about 0.1 and 5% (W/V), preferably about 0.5% (W/V). Suitable, but non-limiting, examples of stabilizing additives include gum acacia, gelatin, methyl cellulose, polyethylene glycol, carboxylic acids and salts thereof, and polylysine. The preferred stabilizing additives are gum acacia, gelatin and methyl cellulose.

[00138] The amount of active agent, e.g., insulin, is an amount effective to accomplish the purpose of the particular active agent. The amount in the composition is a therapeutically effective dose, i.e., a pharmacologically or biologically effective amount. However, the amount can be less than a pharmacologically or biologically effective amount when the composition is used in a dosage unit form, such as a capsule, a tablet or a liquid, because the dosage unit form may contain a multiplicity of delivery agent/biologically or chemically active agent compositions or may contain a divided pharmacologically or biologically effective amount. The total effective amounts can then be administered in cumulative units containing, in total, pharmacologically or biologically or chemically active amounts of biologically or pharmacologically active agent.

[00139] The total amount of active agent, and particularly insulin, to be used can be determined by those skilled in the art. However, it has surprisingly been found that with some biologically or chemically active agents, the use of the presently disclosed delivery agents provides extremely efficient delivery.

[00140] The amount of delivery agent in the present composition is a delivery effective amount and can be determined for any particular delivery agent/active agent combination by methods known to those skilled in the art.

[00141] The oral dosage forms of the present invention, containing a mixture of the active agent, e.g., insulin and the delivery agent, e.g., 4-CNAB or separately containing the active agent and the delivery agent, may include additional materials known to those skilled in the art as pharmaceutical excipients. Any excipient or ingredient, including pharmaceutical ingredients or excipients. Such pharmaceutical excipients include, for example, the following: Acidifying agents (acetic acid, glacial acetic acid, citric acid, fumaric acid, hydrochloric acid, diluted hydrochloric acid, malic acid, nitric acid, phosphoric acid, diluted phosphoric acid, sulfuric acid, tartaric acid); Aerosol propellants (butane, dichlorodifluoro-methane, dichlorotetrafluoroethane, isobutane, propane, trichloromonofluoromethane); Air displacements (carbon dioxide, nitrogen); Alcohol denaturants (denatonium benzoate, methyl isobutyl ketone, sucrose octacetate); Alkalizing agents (strong ammonia solution, ammonium carbonate, diethanolamine, diisopropanolamine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, trolamine); Anticaking agents (see *glidant*); Antifoaming agents

(dimethicone, simethicone); Antimicrobial preservatives (benzalkonium chloride, benzalkonium chloride solution, benzelthonium chloride, benzoic acid, benzyl alcohol, butylparaben, cetylpyridinium chloride, chlorobutanol, chlorocresol, cresol, dehydroacetic acid, ethylparaben, methylparaben, methylparaben sodium, phenol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric nitrate, potassium benzoate, potassium sorbate, propylparaben, propylparaben sodium, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimerosal, thymol); Antioxidants (ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, tocopherols excipient); Buffering agents (acetic acid, ammonium carbonate, ammonium phosphate, boric acid, citric acid, lactic acid, phosphoric acid, potassium citrate, potassium metaphosphate, potassium phosphate monobasic, sodium acetate, sodium citrate, sodium lactate solution, dibasic sodium phosphate, monobasic sodium phosphate); Capsule lubricants (see *tablet and capsule lubricant*); Chelating agents (edetate disodium, ethylenediaminetetraacetic acid and salts, edetic acid); Coating agents (sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methacrylic acid copolymer, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcystalline wax, zein); Colorants (caramel, red, yellow, black or blends, ferric oxide); Complexing agents (ethylenediaminetetraacetic acid and salts (EDTA), edetic acid, gentisic acid ethanolmaide, oxyquinoline sulfate); Desiccants (calcium chloride, calcium sulfate, silicon dioxide); Emulsifying and/or solubilizing agents (acacia, cholesterol, diethanolamine (adjunct), glyceryl monostearate, lanolin alcohols, lecithin, mono- and di-glycerides, monoethanolamine (adjunct), oleic acid (adjunct), oleyl alcohol (stabilizer), poloxamer, polyoxyethylene 50 stearate, polyoxyl 35 caster oil, polyoxyl 40 hydrogenated castor oil, polyoxyl 10 oleyl ether, polyoxyl 20 cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, propylene glycol diacetate, propylene glycol monostearate, sodium lauryl sulfate, sodium stearate, sorbitan monolaurate, soritan monooleate, sorbitan monopalmitate, sorbitan monostearate, stearic acid, trolamine, emulsifying wax); Filtering aids (powdered cellulose, purified siliceous earth); Flavors and perfumes (anethole,

benzaldehyde, ethyl vanillin, menthol, methyl salicylate, monosodium glutamate, orange flower oil, peppermint, peppermint oil, peppermint spirit, rose oil, stronger rose water, thymol, tolu balsam tincture, vanilla, vanilla tincture, vanillin); Glidants and/or anticaking agents (calcium silicate, magnesium silicate, colloidal silicon dioxide, talc); Humectants (glycerin, hexylene glycol, propylene glycol, sorbitol); Plasticizers (castor oil, diacetylated monoglycerides, diethyl phthalate, glycerin, mono- and di-acetylated monoglycerides, polyethylene glycol, propylene glycol, triacetin, triethyl citrate); Polymers (e.g., cellulose acetate, alkyl celloloses, hydroxyalkylcelloloses, acrylic polymers and copolymers); Solvents (acetone, alcohol, diluted alcohol, amylene hydrate, benzyl benzoate, butyl alcohol, carbon tetrachloride, chloroform, corn oil, cottonseed oil, ethyl acetate, glycerin, hexylene glycol, isopropyl alcohol, methyl alcohol, methylene chloride, methyl isobutyl ketone, mineral oil, peanut oil, polyethylene glycol, propylene carbonate, propylene glycol, sesame oil, water for injection, sterile water for injection, sterile water for irrigation, purified water); Sorbents (powdered cellulose, charcoal, purified siliceous earth); Carbon dioxide sorbents (barium hydroxide lime, soda lime); Stiffening agents (hydrogenated castor oil, cetostearyl alcohol, cetyl alcohol, cetyl esters wax, hard fat, paraffin, polyethylene excipient, stearyl alcohol, emulsifying wax, white wax, yellow wax); Suspending and/or viscosity-increasing agents (acacia, agar, alginic acid, aluminum monostearate, bentonite, purified bentonite, magma bentonite, carbomer 934p, carboxymethylcellulose calcium, carboxymethylcellulose sodium, carboxymethycellulose sodium 12, carrageenan, microcrystalline and carboxymethylcellulose sodium cellulose, dextrin, gelatin, guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium aluminum silicate, methylcellulose, pectin, polyethylene oxide, polyvinyl alcohol, povidone, propylene glycol alginate, silicon dioxide, colloidal silicon dioxide, sodium alginate, tragacanth, xanthan gum); Sweetening agents (aspartame, dextrates, dextrose, excipient dextrose, fructose, mannitol, saccharin, calcium saccharin, sodium saccharin, sorbitol, solution sorbitol, sucrose, compressible sugar, confectioner's sugar, syrup); Tablet binders (acacia, alginic acid, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methycellulose, polyethylene oxide, povidone, pregelatinized starch, syrup); Tablet and/or capsule diluents (calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, microcrystalline

cellulose, powdered cellulose, dextrates, dextrin, dextrose excipient, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, compressible sugar, confectioner's sugar); Table disintegrants (alginic acid, microcrystalline cellulose, croscarmellose sodium, corspovidone, polacrilin potassium, sodium starch glycolate, starch, pregelatinized starch); Tablet and/or capsule lubricants (calcium stearate, glyceryl behenate, magnesium stearate, light mineral oil, polyethylene glycol, sodium stearyl fumarate, stearic acid, purified stearic acid, talc, hydrogenated vegetable oil, zinc stearate); Tonicity agent (dextrose, glycerin, mannitol, potassium chloride, sodium chloride); Vehicle: flavored and/or sweetened (aromatic elixir, compound benzaldehyde elixir, iso-alcoholic elixir, peppermint water, sorbitol solution, syrup, tolu balsam syrup); Vehicle: oleaginous (almond oil, corn oil, cottonseed oil, ethyl oleate, isopropyl myristate, isopropyl palmitate, mineral oil, light mineral oil, myristyl alcohol, octyldodecanol, olive oil, peanut oil, persic oil, seame oil, soybean oil, squalane); Vehicle: solid carrier (sugar spheres); Vehicle: sterile (bacteriostatic water for injection, bacteriostatic sodium chloride injection); Viscosity-increasing (see *suspending agent*); Water repelling agent (cyclomethicone, dimethicone, simethicone); and Wetting and/or solubilizing agent (benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, docusate sodium, nonoxynol 9, nonoxynol 10, octoxynol 9, poloxamer, polyoxyl 35 castor oil, polyoxyl 40, hydrogenated castor oil, polyoxyl 50 stearate, polyoxyl 10 oleyl ether, polyoxyl 20, cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, sodium lauryl sulfate, sorbitan monolaureate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, tyloxapol). This list is not meant to be exclusive, but instead merely representative of the classes of excipients and the particular excipients which may be used in oral dosage forms of the present invention.

[00142] In the case of insulin, oral delivery may have advantages beyond convenience, acceptance and compliance issues. Insulin absorbed in the gastrointestinal tract mimics the physiology of insulin secreted by the pancreas because both are released into the portal vein and carried directly to the liver. Absorption into the portal circulation maintains a peripheral-portal insulin gradient that regulates insulin secretion. In its first passage through the liver, roughly 60% of the insulin is retained and metabolized, thereby reducing the incidence of peripheral

hyperinsulinemia, a factor in diabetes related systemic complications. A feared and not uncommon complication of insulin treatment and other oral antidiabetic agents is hypoglycemia.

[00143] The present invention relates in part to a method of treating human diabetics via the chronic oral administration of insulin together with a drug delivery agent that enhances the absorption of insulin (e.g., from the duodenum) such that a therapeutically effective control and/or reduction in blood glucose is achieved while effecting a reduction in the systemic blood insulin concentration (serum insulin level) on a chronic basis required to achieve the reduction in blood glucose concentration, e.g., relative to the serum insulin level required to achieve therapeutic efficacy via subcutaneous injection of insulin.

[00144] Whereas traditional subcutaneous insulin dosing shifts the point of entry of insulin into the circulation from the natural site (the portal vein) to the systemic circulation, the oral dosing method of the present invention shifts the site of insulin entry back to the portal vein. The effect of this route of dosing is two fold. First, by targeting the liver directly, a greater control of glucose may be achieved. Various studies have shown that intraportal delivery of insulin can yield a comparable control of glucose at infusion rates lower than those required by peripheral administration. (Stevenson, R. W. et al., Insulin infusion into the portal and peripheral circulations of unanaesthetized dogs, Clin Endocrinol (Oxf) 8, 335-47 (1978); Stevenson, R. W. at al., Effect of intraportal and peripheral insulin on glucose turnover and recycling in diabetic dogs, Am J Physiol 244, E190-5 (1983); Shishko, P. I. et al., I. U. Comparison of peripheral and portal (via the umbilical vein) routes of insulin infusion in IDDM patients, Diabetes 41, 1042-9 (1992). Because the insulin will undergo first-pass metabolism prior to entering the systemic circulation, a lower serum concentration is achieved. This may, in turn, alleviate any detrimental effects of insulin on non-target tissues.

[00145] In normal healthy humans, the physiologic ratio of blood insulin concentration in the portal vein as compared to systemic (peripheral) blood insulin concentration is greater than about 2:1. In contrast, administration of insulin to human diabetic patients has been found to shift this ratio of portal vein insulin blood concentration to systemic insulin blood concentration to about 0.75:1. By virtue of the present invention, the ratio of concentration of unmodified insulin in the

portal circulation to systemic circulation approaches the normal physiological ratio, e.g., from about 2:1 to about 6:1.

[00146] In some embodiments, the invention provides a method of treating diabetic patients, comprising orally administering an oral insulin treatment comprising a dose of insulin together with a delivery agent that facilitates the absorption of said insulin from the gastrointestinal tract on a chronic basis to diabetic patients to reduce blood glucose levels in said diabetic patients by a desired amount, such that the concentration of insulin circulating in the blood of said diabetic patients as a result of said oral insulin treatment is not substantially greater than normal physiological levels.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[00147] In order that this invention may be better understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any manner.

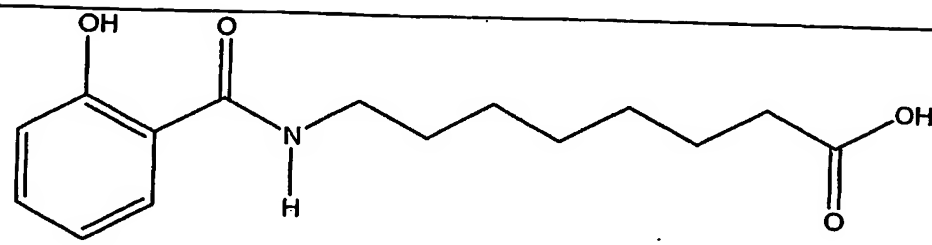
EXAMPLE 1

Plasma Delivery Agent Design and Efficiency

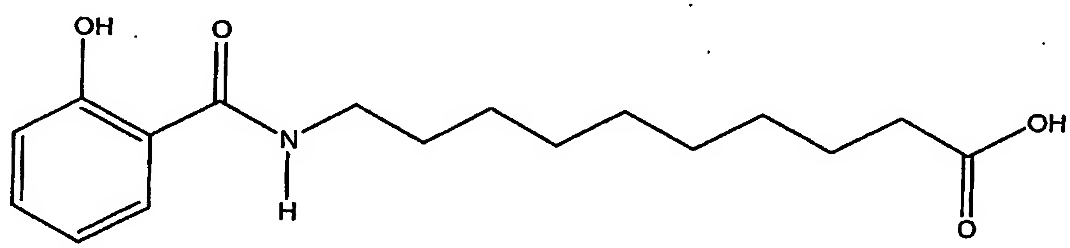
[00148] Delivery agents 1-3 were investigated for their ability to penetrate the GI mucosa. The plasma concentration of each delivery agent was measured in human subjects after oral administration of delivery agent loaded capsules as a measure of each delivery agent's penetration efficiency. See Tables 1 and 2.

Table 1: Structures of Delivery Agents 1-3

Delivery Agent 1 (SNAC)



Delivery Agent 2 (SNAD)



Delivery Agent 3 (4-CNAB)

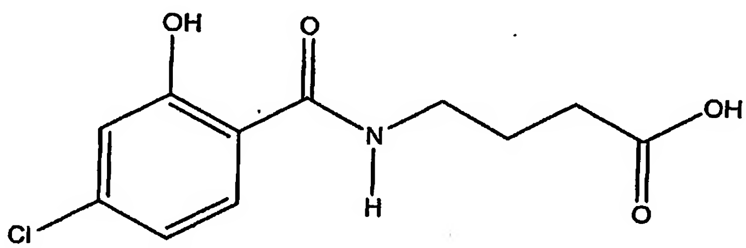


Table 2: Delivery Agent Plasma Concentrations in Humans

Delivery Agent	Variables		Delivery Agent Dose (Mg)	AUC (ng.hr/ml)
	X	n		
1 (SNAC)	H	7	750	3499
2 (SNAD)	H	9	750	2037
3 (4-CNAB)	Cl	3	800	47478

[00149] Blood sampling for plasma delivery agent concentration determination (2 mL in sodium heparin tube) were drawn 15 minutes before dosing, and at 5, 10, 15, 30, and 45 minutes and 1,

1.5, 2, 3, 4, 6, 8, and 12 hours post-dose (14 samples per treatment) for delivery agent measurements in all treatment groups.

[00150] Two 18-gauge IV lines were situated prior to dosing; one for blood sampling, and the other for potential infusion of 20% glucose for subjects in groups 2 and 3. The subjects in group 1 only had one cannula inserted. The blood samples were centrifuged at 3000 rpm for a period of fifteen minutes at a temperature from about 2°C to 8°C, within one hour of sample collection. Using a plastic pipette and without disturbing the red cell layer, the plasma from the collection tube was pipetted in duplicate for each analysis, blood glucose, Human Insulin, C-peptide, delivery agent into pre-labeled polypropylene tubes. The samples were stored at –70 ^{0}C until analysis.

[00151] The indicated doses were ingested by healthy human volunteers and the plasma concentrations of the delivery agents were monitored over time and the area under the curve (AUC) calculated. Surprisingly, as provided in Table 2, oral administration of 800 mg delivery agent number 3 with X as chlorine and n equal to 3 alkyl produced an approximately 13.5 fold greater penetration of the GI mucosa in humans than did oral administration of 750 mg of delivery agent 1 having n equal 7 alkyl. Similarly, oral administration of 800 mg of delivery agent number 3 produced more than a 23 fold greater penetration of the GI mucosa in humans than did oral administration of 750 mg of delivery agent 2 having n equal to 9 alkyl.

[00152] Similar results were obtained when delivery agents 1-3 were administered orally to monkeys and the plasma concentrations of the delivery agents monitored over time and the AUC calculated. As provided in Table 3, oral administration of 300 mg of delivery agent number 3 with X as chlorine and n equal to 3 alkyl produced a more than 11 fold greater penetration of the GI mucosa in monkeys than did oral administration of 300 mg of delivery agent 1 having n equal to 7 alkyl. See Table 3. Further, 300 mg of delivery agent 3 displayed a more than 6 fold greater penetration of the GI mucosa in monkeys than did oral administration of 300 mg of delivery agent 2 having n equal to 9 alkyl. See Table 3.

Table 3: Delivery Agent Plasma Concentrations in Monkeys

Delivery Agent			Delivery Agent Dose (Mg)	AUC (ng.hr/ml)
	X	n		
1 (SNAC)	H	7	300	45
2 (SNAD)	H	9	300	82
3 (4-CNAB)	Cl	3	300	499

EXAMPLE 2

Comparison of the Delivery Efficiency of Delivery Agents 1-3

[00153] Next, delivery agents 1-3 were compared for the ability to efficiently transport an active agent across the GI mucosa in a biologically active form by determining the relationship between delivery agent dose, dose of active agent and the glucose response. See Table 4. The effective dose of delivery agent necessary to deliver a therapeutic dose of active agent and produce a therapeutic effect was measured. See Table 4. For delivery agent 3, the active agent was insulin, and the therapeutic effect was determined by the ability of the delivery agent/insulin combination to lower serum glucose by at least 10% within one hour post administration. For delivery agents 1 and 2, the active agent was heparin, and the therapeutic effect was determined by **[Emisphere: please fill in]**

Table 4: Effective Clinical Dose of Delivery Agent in Humans

Delivery Agent	X	N	Delivery Agent Dose (Mg)
1 (SNAC)	H	7	2400
2 (SNAD)	H	9	1500
3 (4-CNAB)	Cl	3	200

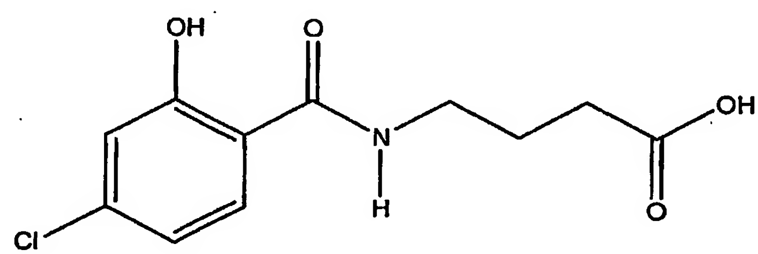
[00154] Again, as shown in Table 4, delivery agent 3 with X as chlorine and n equal to 3 alkyl was approximately 12 fold more efficient in facilitating insulin transit across the GI mucosa in a biologically active form than was delivery agent 1 having n equal to 7 alkyl. Similarly, delivery agent no. 3 was 7.5 fold more efficient in facilitating transport of insulin across the GI mucosa in a biologically active form than was delivery agent 2 having n equal to 9 alkyl. See Table 4.

[00155] Most importantly, only delivery agent 3 is efficient enough at facilitating transport of biologically active insulin to allow packaging of a therapeutically effective dose of insulin plus delivery agent into a single capsule.

EXAMPLE 3

Preparation of the Delivery Agent 4-CNAB

[00156] The compound corresponding to the following structure may be prepared as described below:



[00157] 4-Chlorosalicylic acid (10.0g, 0.0579 mol) was added to a one-neck 250 ml round-bottomed flask containing about 50 ml methylene chloride. Stirring was begun and continued for the remainder of the reaction. The coupling agent 1,1-carbonyldiimidazole (9.39g, 0.0579 mol) was added as a solid in portions to the flask. The reaction was stirred at room temperature for approximately 20 minutes after all of the coupling agent had been added and then ethyl-4-aminobutyrate hydrochloride (9.7 g , 0.0579 mol) was added to the flask with stirring. Next, triethylamine (10.49 ml, 0.0752 mol) was added dropwise from an addition funnel. The addition funnel was rinsed with methylene chloride. The reaction was allowed to stir at room temperature overnight.

[00158] The reaction was poured into a separatory funnel and washed with 2N HCl and an emulsion formed. The emulsion was left standing for two days and was then filtered through celite in a fritted glass funnel. The filtrate was put back in a separatory funnel to separate the layers. The organic layer was dried over sodium sulfate, which was then filtered off and the filtrate concentrated by rotary evaporation. The resulting solid material was hydrolyzed with 2N NaOH, stored overnight under refrigeration, and then hydrolyzing resumed. The solution was

acidified with 2N HCl and the solids that formed were isolated, dried under vacuum, and recrystallized twice using methanol/water. Solids precipitated out overnight and were isolated and dried. The solids were dissolved in 2N NaOH and the pH of the sample was brought to pH 5 with 2N HCl. The solids were collected and HPLC revealed a single peak. These solids were then recrystallized in methanol/water, isolated, and then dried under vacuum, yielding 4.96g (33.0%) of 4-(4 chloro-2-hydroxybenzoyl)aminobutyric acid, ($C_{11}H_{12}ClNO_4$; Molecular weight 257.67). A melting point of 131-133 °C was determined. Combustion analysis revealed the following content: %C: 51.27(calc.), 51.27 (found); %H: 4.69 (calc.), 4.55 (found); %N: 5.44 (calc.), 5.30 (found). Proton H NMR Analysis revealed: (d_6-DMSO): d 13.0, s, 1H (COOH); d 12.1, s, 1H (OH); d 8.9, t, 1H (NH); d 7.86, d, 1H (H ortho to amide); d 6.98, d, 1H (H ortho to phenol OH); d 6.96, d, 1H, (H meta to amide); d 3.33, m, 2H (CH_2 adjacent to NH); d 2.28, t, 2H (CH_2 adjacent to COOH); d 1.80, m, 2H (aliphatic CH_2 beta to NH and CH_2 beta to COOH).

<u>4-CNAB Preparation for Human Studies</u>

[00159] 4-CNAB for the human dosings (Monosodium N-(4-chlorosalicyloyl)-4-amino-butyrate) was made under good manufacturing practices (GMP) conditions by Regis Technologies, Inc. (Morton Grove, IL) according to the methods of International Publication No. WO 00/46182 except that the starting material 4-chlorosalicylic acid (purchased from Ihara Chemical Industry Co. Inc, Ltd., Tokyo, Japan and Aapin Chemicals Ltd., Oxfordshire, UK) was used and converted to the amide via a methyl ester using 0.14 equivalents sulfuric acid in methanol and then about 4 equivalents ammonia in methanol. The alkylating agent used was ethyl-4-bromobutyrate.

[00160] The monosodium salt of 4-CNAB was made according to the following method on a 40 kilogram scale. 4-CNAB free acid (500 g, 1.94 mol, FW = 257.67) was charged to a 22 L five neck round bottom flask. The flask was equipped with an overhead stirrer, a thermocouple temperature read out, a reflux condenser and a heating mantle, and was placed under nitrogen. Reagent grade acetone (13 L) was added to the reactor and the mixture was agitated. The 4-CNAB/acetone mixture was heated to 50° C to dissolve any solids. A hazy brown solution was achieved.

[00161] The 50° C solution was pumped through a warm pressure filter (dressed with Whatman #1 filter paper, ~5 microns, 18.5 sq. in. area) into a clean 22 L reactor to remove sodium chloride and other insolubles. The pressure dropped across the filter to about 20 psig at the end of filtration. The reactor containing the clear yellow filtrate was agitated and heated. At 50° C the reactor was removed from heat.

[00162] The clear filtrate was charged with 50% sodium hydroxide solution (155 g, 1.94 mol) as rapidly as possible, while maintaining a vigorous agitation. (An overcharge will result in the undesirable formation insoluble disodium salts. A slight undercharge is preferable because the free acid is removed during the final filtration step.) The reaction mixture exothermed to approximately 52° C. Precipitates formed and the product gelled before becoming clear again.

[00163] After the base addition was completed and the temperature leveled, the solution became cloudy and increased in viscosity. The reaction was refluxed for 2 hours at 60° C, while agitating vigorously. The reaction mixture continued to thicken, forming solid chunks. The slurry became light pink and foamed. The reactor contents were cooled to ambient temperature over 3 to 4 hours. The ambient temperature was held for 30 minutes. The precipitated solids were isolated on a filter funnel. The isolated product was not washed. The resulting 4-CNAB monosodium salt was dried *in vacuo* at 40 to 50° C for 16 to 24 hours to give 490 grams (1.75 mol, 90% yield, FW = 279.65).

[00164] The insulin for the subcutaneous injection was HUMULIN® R injection insulin from Eli Lilly and Company (Indianapolis, IN).

[00165] All capsules containing 200 mg 4-CNAB and 150 insulin units USP were prepared as follows. First, the total amount of delivery agent material necessary for filling the delivery agent alone capsules and the delivery agent plus insulin composition capsules was prepared by weighing 3160 g of 4-CNAB. The 3160 g 4-CNAB was then milled in a Quadro *comil, model 197S* mill with screen number 2A 050 G 037 19 136 (1270 micron). Next, 1029 g of the milled 4-CNAB was passed through a #35 mesh screen. Then, the pass through screened material was transferred into a 4 quart shell and blended using for example, a V blender, at 25 rpm for 10.2 minutes. The resultant blended material was used to fill capsules. In this case, a Fast Cap

Capsule Filler was used with a size 3 Fast Cap Encapsulation tray. The empty capsules weighed approximately 48 mg each and were filled with an average fill weight of 205.6 mg of 4-CNAB alone. Thus, the dose of the delivery agent alone capsules was 205.6 mg.

[00166] The insulin compositions were prepared by first dispensing 31.8 g of recombinant human zinc crystalline insulin (Potency 26.18 Units per mg) (proinsulin derived (recombinant DNA origin) USP quality) from Eli Lilly and Company (Indianapolis, IN) into an appropriately sized plastic bag. Next, sequential 30 g additions of the milled and screened 4-CNAB were added to the bag until approximately 510 g had been added. The bag was thoroughly mixed after each 30 g addition of 4-CNAB by shaking and inversion. In order to add and mix the next 532.5 g of 4-CNAB, the 541.8 g mixture of insulin and 4-CNAB was transferred to a V blender and mixed again at 25 rpm for 10.2 minutes. Next, the remaining 4-CNAB was added to the blender and the entire mixture was mixed in the blender at 25 rpm for 10.2 minutes. Finally, the resulting composition was dispensed as described above into empty capsules. The final capsules contained an average of 5.7 mg insulin (equivalent to 150 units insulin) and 200.5 mg of 4-CNAB or a ratio of 1:57.3, insulin: 4-CNAB. Multiple samples of the final blend were run on HPLC to verify uniformity and were found to be uniform.

EXAMPLE 4

<u>Previous Non-clinical Studies with 4-CNAB and Insulin/4-CNAB</u>

[00167] The present invention comprising compositions of insulin and the delivery agent 4-CNAB was evaluated for safety and toxicity in a nonclinical program that included pharmacological screening, pharmacokinetic profiling, and toxicity assessments in rats and monkeys. In general, animal physiological responses to 4-CNAB alone and to Insulin/4-CNAB were comparable. Pharmacokinetic studies in mice, rats and monkeys have shown that 4-CNAB is absorbed rapidly following oral administration, and subsequently cleared from the body. 4-CNAB did not demonstrate potential activity in any of the primary molecular targets evaluated in receptor binding screening assays. Four genotoxicity studies have been conducted with 4-CNAB, with no positive findings. Based on 14-day oral repeated dose toxicity studies, the

NOAEL (No-Adverse Effect Level) was estimated to be 500 mg/kg in Sprague-Dawley rats, and 400 mg/kg in rhesus monkeys.

[00168] In toxicology studies, 4-CNAB doses from 400 mg to 2000 mg were evaluated. Following 14-day oral repeated dose toxicity studies in rats and monkeys, the estimated No Adverse Effect Level (NOAEL) for 4-CNAB was 500 mg/kg in Sprague-Dawley rats and 400 mg/kg in rhesus monkeys; therefore, the monkey appeared to be the most sensitive species. The highest proposed dose of 2000 mg 4-CNAB in man (<30 mg/kg) is 12-16 fold lower than the NOAEL in monkeys (i.e., NOAEL = 400 mg/kg 4-CNAB alone and in combination with 15 U/kg insulin). The absolute bioavailability of insulin in monkeys was about 1% or less. In the toxicology studies, there were no findings in rats attributed to insulin at an oral dose level of 15 U/kg in combination with 4-CNAB doses as high as 2000 mg/kg. In monkeys, an insulin dose of 15 U/kg was associated with a single hypoglycemic episode in combination with a 4-CNAB dose of 1200 mg/kg in one monkey; there were no effects at 15 U/kg insulin in combination with lower doses.

[00169] Non-clinical studies in rats and monkeys demonstrated that, over the range tested, insulin absorption increases with increasing doses of 4-CNAB. Similarly, for a fixed oral dose of 4-CNAB, insulin absorption increases with increasing doses of insulin. Oral insulin absorption was evaluated in rats at varying doses of both insulin and 4-CNAB. Significant increases in serum insulin concentrations were observed following the administration of insulin at doses of 4.55, 6.5, 9.75, and 13 Units/kg in the presence of a fixed 4-CNAB dose (200 mg/kg). The mean peak serum insulin levels were 31, 44, 85, and 132 μU/mL respectively. Insulin absorption was dose dependent and increased as the dose of insulin increased. Oral administration of aqueous solutions of insulin alone (13 Units/kg) or 4-CNAB alone (200 mg/kg) did not result in any significant increases in serum insulin levels. Significant increases in serum insulin concentrations were also observed following the administration of 4-CNAB at doses of 50, 100, 200, and 300 mg/kg in the presence of a fixed insulin dose (13 Units/kg). The mean peak serum insulin levels were 9, 39, 103, and 157 μU/mL, respectively. Insulin absorption was dose dependent and increased as the dose of 4-CNAB increased.

[00170] Based on the above nonclinical information, the starting insulin dose of 150 insulin

Units USP (which is about 7-fold lower than the 15 U/kg no effect dose in monkey) was selected.

EXAMPLE 5

[00171] In this example, the oral insulin capsule(s) described herein were orally administered to twenty human subjects with diabetes at night before going to sleep.

[00172] The rationale for nighttime administration is as follows. The clinical studies reported herein with oral insulin in type 2 diabetic patients demonstrated a hypoglycemic effect of short duration. This probably indicates that the half-life of systemic circulating insulin provided by oral insulin is short to affect peripheral glucose disposal. It was hypothesized that orally administered insulin as set forth herein may, however, have a more profound effect on hepatic glucose production due to portal delivery. Hepatic glucose production is responsible for the fasting blood glucose levels.

[00173] In type 2 diabetics, blood glucose levels are often elevated after an overnight fast, presumably because of unrestrained glucose production by the liver as a result of a combination of insulin resistance and insufficient insulin secretion, which is the hallmark of the disease. Elevated blood glucose levels can lead to a vicious cycle to perpetuate the severity of a diabetic's condition because, if blood glucose is elevated for an extended period of time, a corresponding "wear and tear" on the cells in the pancreas that secrete insulin to regulate blood glucose levels is possible. Thus, if a treatment were to spare insulin producing cell function, this "rest" to the cells may provide for long-term protection to develop overt diabetes. It was therefore proposed to study the effect of bedtime oral insulin on hepatic glucose production and hence FBG (free blood glucose).

[00174] In accordance with the above, this example reports the results of an open-label, single-dose, crossover study comparing the safety of orally administered 4-CNAB/Insulin formulation with that of subcutaneously injected insulin in two groups of subjects with type 2 diabetes mellitus -- one in the fasting state and one with a standard meal. The objectives were (1) to compare the safety, pharmacokinetics and pharmacodynamics of orally administered 4-CNAB/insulin with that of subcutaneously injected regular insulin in fasting type 2 diabetic

subjects, and (2) to compare blood glucose, insulin and C-peptide levels after a standard meal with regular medication with blood glucose, insulin and C-peptide levels after a standard meal with 4-CNAB/insulin.

[00175] The focus of this example is the assessment of the safety of insulin/4-CNAB, administered orally at bedtime, to type 2 diabetic subjects. The purpose of the study was to determine if the administration of oral insulin at bedtime could exert effects on overnight-fasting glucose homeostatsis and insulin secretion. The postulated mode of action (e.g., suppressing the liver production of glucose, and thus preventing beta cell death or dysfunction of insulin producing) was the basis for the design of the study.

[00176] Twenty-four human subjects (patients) of age 35-70 years with elevated fasting blood glucose levels (type 2 diabetes), but in otherwise good general health on the basis of a medical history, physical examination, clinical laboratory studies, participated in the study and were studied in the overnight-fasted state on two occasions, separated by an interval of at least 7 days. The following treatment conditions were studied:

Group 1: twelve (12) type 2 diabetic subjects: (a) oral insulin/4-CNAB – fasted subjects, and (b) empty capsule – fasted subjects.

Group 2: twelve (12) type 2 diabetic subjects: (a) standard meal with regular medication, and (b) standard meal with oral human insulin/4-CNAB.

[00177] A total of twenty subjects participated in the second part of the study, relating to the safety of insulin/4-CNAB administered orally at bedtime, an additional four subjects not being included due to logistical considerations. These twenty subjects took an oral insulin capsule(s) at night before going to sleep. The trial took place at the home of the subject under the supervision of a bedside private duty nurse. The rationale to conduct the trial at the patient own environment was based on the fact that glucose homeostasis is best reflected when conducted in a familiar environment and changes significantly with hospitalization.

[00178] Fasting blood glucose, insulin and C-peptide levels were measured at 7:00 a.m. for three days to establish baseline levels. On two successive nights and mornings before taking the capsule, the subjects measured their glucose levels with a glucometer (supplied). If the subject's

glucose levels were >120 mg/dl on the first two mornings (fasting), on the 3rd night, the subject took the insulin capsule(s). If, on the first two successive mornings, the patient's fasting blood glucose was not greater than 120 mg/dl, then the patient was dismissed from the study and all final study procedures were performed as per the protocol. The subjects ate their regular dinner at home, as every evening, between the hours of 7:00 and 8:00 P.M. If the subjects usually took medication for the diabetes (metformin or acarbose) in the evening, they took their usual dose.

[00179] At 11:00 p.m. (at least two hours after dinner), the subjects took one oral insulin dose that contained the following ingredients: 300 mg 4-CNAB and insulin according to the dose (200-400 U) that the subject received during the first phase of the trial. If the subject had received 200 U insulin in the first phase of the trail and there was no drop in blood glucose level (<15% reduction), he now received 300 U of insulin. If the subject had received 300 U insulin in the first phase of the trail and there was no drop in blood glucose level (<15% reduction), he now received 400 U of insulin. None of the subjects received more than 400 U of insulin. The capsules were prepared by AAI and have shown stability.

[00180] A nurse was present at the home of the subjects when they took the oral insulin capsules and throughout the night. The nurse checked the blood glucose level with a glucometer before the subjects took the medication. In addition, blood was taken for further blood glucose levels, insulin and C-peptide. Orange juice was readily available for treatment in the unlikely event of hypoglycemia. During sleep the subjects wore a Glucowatch (which is a monitor of blood glucose and measures and records blood glucose levels at regular intervals). The Glucowatch is equipped with an alarm triggered when blood glucose levels reach predetermined blood glucose levels (hypoglycemic levels) determined by the investigator or patient. The bedside private duty nurse was also present during the night to monitor the patient. In the morning, when the subjects woke up (e.g., at 7:00 a.m.), the nurse checked their blood glucose level with the glucometer. Additional blood samples were taken for further blood glucose levels, insulin and C-peptide. The blood samples from the night before were stored in the refrigerator at home and in the morning the nurse brought the samples of blood (from the night and the morning) to the lab for analysis.

[00181] There were no serious adverse effects in the course of the study. The results of the nighttime oral insulin study reported as the example herein (fasting blood glucose, insulin and C-

peptide measured at approximately 7:00 a.m. and compared to the patient's own baseline levels) are set forth in Figures 1-4. The results are reported by patient with numeric values in Figure 1 (μUU/ml), and are graphically represented in Figures 2-4.

[00182] The overnight study demonstrated that the metabolic effect of a single dose of oral insulin was still apparent in the morning, i.e., about eight hours after administration. As a result, there was a decrease in blood glucose output from the liver. As shown in Figure 2 (effect on blood glucose), there was no statistically significant difference between the baseline blood glucose levels and the blood glucose levels in the patients after administration of the nighttime oral insulin capsules. Blood glucose measured the morning after administration decreased by 6% from baseline levels, i.e., from 133.78 ± 40.53 mg/dl to 125.78 ± 42.99 ($p=0.017$).

[00183] On the other hand, in all patients, a statistically significant reduction in C-peptide and insulin was detected in the morning (while the glucose levels were somewhat unchanged). A consistent compensatory decline in C-peptide levels from baseline by a mean of 24%, i.e., from 2.69 ± 0.88 ng/ml to 2.04 ± 0.71 ($p<0.001$) indicated that there was less activity in the beta cells that secrete endogenously produced insulin. Plasma insulin levels were reduced by a mean of 33%, i.e., from 13.90 ± 6.44 μU/ml to 9.35 ± 4.52 ($p<0.001$). These results are graphically depicted in Figures 3 and 4, respectively.

[00184] The interpretation of these results is that a "boost" of exogenous insulin at nighttime allows the patients' beta cells to rest and produce less insulin to achieve the same glycemic level. The suggested clinical implication is that if such treatment were to be given (bed time oral insulin) alone, it is likely to spare beta cell function as these cell become dysfuctional or die from exhaustion. This significance is supported by several reported studies which have shown that by intervening "aggressively" with insulin at early stages of the disease (such as IGT or "impaired glucose tolerance" stage), by giving insulin even for a short time such as two week duration, that this "rest" to the cells may provide for long term protection to develop overt diabetes.

[00185] It was further seen in this study that none of the patients had a clinically significant hypoglycemic episode, despite that the insulin was administered to the patients in the fasting state and with continued fasting. This result supports the conclusion that the administration of

oral insulin formulations as described herein will be safe in terms of hypoglycemia.

[00186] In the preceding specification, the invention has been described with reference to specific exemplary embodiments and examples thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention as set forth in the claims that follow. The specification and drawings are accordingly to be regarded in an illustrative manner rather than a restrictive sense.

[00187] While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic constructions can be altered to provide other embodiments which utilize the processes and compositions of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than by the specific embodiments which have been presented hereinbefore by way of example.

WHAT IS CLAIMED IS:

1. A method for prophylactically sparing beta cell function in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime.

2. A method for preventing beta cell death or dysfunction in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime.

3. A method for long term protection of a mammal which has impaired glucose tolerance or early stage diabetes mellitus from developing overt diabetes, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime.

4. A method for delaying the onset of overt diabetes in a mammal which has impaired glucose tolerance or early stage diabetes mellitus, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime.

5. The methods of claims 1, 2, 3 or 4, wherein the mammal is a human.

6. The methods of claims 1, 2, 3, 4 or 5, wherein the oral pharmaceutical formulation of insulin is administered at bedtime.

7. The method of claims 1, 2, 3, 4, 5 or 6, wherein the oral pharmaceutical formulation is administered on a chronic basis.

8. The method of claims 1, 2, 3, 4, 5 or 6, wherein the oral pharmaceutical formulation is administered nightly for at least two weeks.

9. The method of claim 5, which provides a lowering of insulin of at least about 20%.

10. The method of claim 5, which achieves a therapeutically effective reduction in blood glucose after oral administration to a human diabetic patient, and which provides a ratio of portal vein to peripheral blood insulin concentration from about 2.5:1 to about 6:1.

11. The method of claim 5, wherein the oral dosage form of claim 3, wherein said dosage form is solid.

12. The method of any of the foregoing claims, wherein the dose of insulin contained in the dosage form is from about 50 Units to about 600 Units (from about 2 to about 23mg).

13. The method of any of the foregoing claims, wherein the dose of unmodified insulin is from about 100 Units (3.8 mg) to about 400 Units (15.3 mg) insulin.

14. The method of any of the foregoing claims, wherein the dose of unmodified insulin is from about 150 Units (5.75 mg) to about 300 Units (11.5 mg).

15. The method of any of the foregoing claims, wherein the dosage form(s) begin delivering insulin into the portal circulation (via absorption through the mucosa of the stomach) to achieve peak levels within about 30 minutes or less.

16. A method of treating humans having impaired glucose tolerance or early stage diabetes mellitus, comprising,

orally administering insulin before bedtime to humans having impaired glucose tolerance or early stage diabetes mellitus such that a statistically significant decrease in C-peptide levels from a mean baseline level is achieved in said humans when said C-peptide level is measured about 8 hours after said oral administration of insulin.

17. The method of claim 16, wherein said C-peptide levels when measured are decreased by a mean of about 24%.

18. The method of claim 16 or 17, wherein plasma insulin levels are reduced by a statistically significant degree from baseline when measured about 8 hours after said oral administration of insulin.

19. The method of claim 18, wherein said plasma insulin levels are reduced by a mean of about 33% from baseline when measured about 8 hours after said oral administration of insulin.

20. The method of claim 16, 18 or 19, wherein blood glucose levels are reduced by a insignificant degree from baseline when measured about 8 hours after said oral administration of insulin.

21. The method of claim 20, wherein said blood glucose levels are reduced by a mean of about 6% from baseline when measured about 8 hours after said oral administration of insulin.

22. The method of any of claims 16-21, wherein said oral administration of insulin comprises a dose of from about 200 to about 400 units of insulin and an effective amount of a pharmaceutically acceptable delivery agent which facilitates absorption of said insulin from the gastrointestinal tract of said humans.

23. The method of claim 22, wherein said pharmaceutically acceptable delivery agent comprises 4-CNAB.

24. The method of claim 22, wherein said pharmaceutically acceptable delivery agent comprises about 300 mg 4-CNAB.

25. The method of any of claims 16-22, wherein said insulin is an unmodified insulin.

26. A method of prolonging the effect of an oral administration of an unmodified insulin in order to treat human diabetic patents, comprising orally administering at bedtime a dosage form comprising a orally therapeutically effective amount of unmodified insulin to a human diabetic patient which provides an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after said oral administration, such that a statistically significant decrease in C-peptide levels from baseline is achieved in said humans when said C-peptide level is measured about 8 hours after said oral administration of insulin.

27. A method of prolonging the effect of an oral administration of an unmodified insulin in order to treat humans who have impaired glucose tolerance, comprising orally administering at bedtime a dosage form comprising a orally therapeutically effective amount of unmodified

insulin to a human diabetic patient which provides an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after said oral administration, such that a statistically significant decrease in C-peptide levels from baseline is achieved in said humans when said C-peptide level is measured about 8 hours after said oral administration of insulin.

28. A method of prolonging the effect of an oral administration of an unmodified insulin in order to treat human diabetic patents, comprising orally administering at bedtime a dosage form comprising a orally therapeutically effective amount of unmodified insulin to a human diabetic patient which provides an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after said oral administration, such that plasma insulin levels are reduced by a statistically significant degree from baseline when measured about 8 hours after said oral administration of insulin.

29. A method of prolonging the effect of an oral administration of an unmodified insulin in order to treat humans who have impaired glucose tolerance, comprising orally administering at bedtime a dosage form comprising a orally therapeutically effective amount of unmodified insulin to a human diabetic patient which provides an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after said oral administration, such that plasma insulin levels are reduced by a statistically significant degree from baseline when measured about 8 hours after said oral administration of insulin.

30. A method of prolonging the effect of an administration of oral insulin for a time period longer than the time period that the insulin can be measured in the blood stream, comprising orally administering at bedtime a dosage form comprising a orally therapeutically effective amount of insulin to a human diabetic patient which provides an insulin t_{max} at a time point from about 0.25 to about 1.5 hours after said oral administration, such that:

(i) the blood glucose level of the patient when measured about 8 hours after said oral administration is insignificantly changed from baseline levels for all patients; or

(ii) endogenous insulin production of the patient is lowered by a statistically significant degree as compared to baseline insulin levels in all patients when measured about 8 hours after said oral administration, as evidenced by reduced C-peptide levels; or

(iii) plasma insulin levels of the patient are lowered by a statistically significant degree as compared to baseline plasma insulin levels in all patients when measured about 8 hours after said oral administration; or

(iv) any combination of (i) – (iii) above.

31. The method of any of claims 27-30, wherein said oral administration of insulin comprises a dose of from about 200 to about 400 units of insulin and an effective amount of a pharmaceutically acceptable delivery agent which facilitates absorption of said insulin from the gastrointestinal tract of said humans.

32. The method of claim 31, wherein said pharmaceutically acceptable delivery agent comprises 4-CNAB.

33. The method of claim 31, wherein said pharmaceutically acceptable delivery agent comprises about 300 mg 4-CNAB.

34. The method of any of claims 27-30, wherein said insulin is an unmodified insulin.

35. The method of claim 30, wherein said C-peptide levels are decreased by a mean of about 24% when measured about 8 hours after said oral administration of insulin.

36. The method of claim 30, wherein said plasma insulin levels are reduced by a mean of about 33% when measured about 8 hours after said oral administration of insulin.

37. The method of claim 30, wherein said blood glucose levels are reduced by a mean of about 6% when measured about 8 hours after said oral administration of insulin.

ABSTRACT OF THE DISCLOSURE

A method for long-term protection of a mammal which has impaired glucose tolerance or early stage diabetes mellitus from developing overt diabetes, comprising administering an orally effective dose of a pharmaceutical formulation comprising insulin at nighttime, is disclosed.

	C-peptide		Insulin		Glucose	
Subject #	Control	pm insulin	Control	pm insulin	control	pm insulin
1	3.6	1.3	20.0	7.0	117	93
2	3.1	2.3	24.0	12.0	136	179
3	3.4	2.6	11.8	10.0	104	87
4	2.7	2.0	8.8	7.0	117	96
5	2.5	2.5	6.5	5.0	221	207
6	2.1	1.6	7.0	5.0	210	226
7	1.8	1.6	11.0	7.0	78	100
8	1.9	2.0	8.8	8.0	199	137
9	3.8	2.8	16.5	16.0	112	126
10	0.9	1.2	5.0	6.0		
11	2.7	2.2	17.0	12.0		
12	3.0	1.4	13.0	7.0	125	107
13	2.9	2.3	18.0	10.0	124	103
14	1.4	1.3	9.3	5.0	123	104
15	2.6	1.3	17.0	5.0	93	78
16	4.9	3.3	29.0	23.0	156	173
17	2.6	2.0	19.8	12.0	144	125
18	2.3	1.3	16.0	8.0	142	121
19	2.6	1.9	14.0	8.0	84	84
20	2.9	3.8	5.5	14.0	123	118
Average	2.69	2.04	13.90	9.35	133.78	125.78
Std Dev	0.88	0.71	6.44	4.52	40.53	42.99
Std Error Mean	0.20	0.16	1.44	1.01	9.55	10.13
Ttest	0.00079		0.00073		0.16806	
	0.00039		0.00036		0.08403	

Figure 1

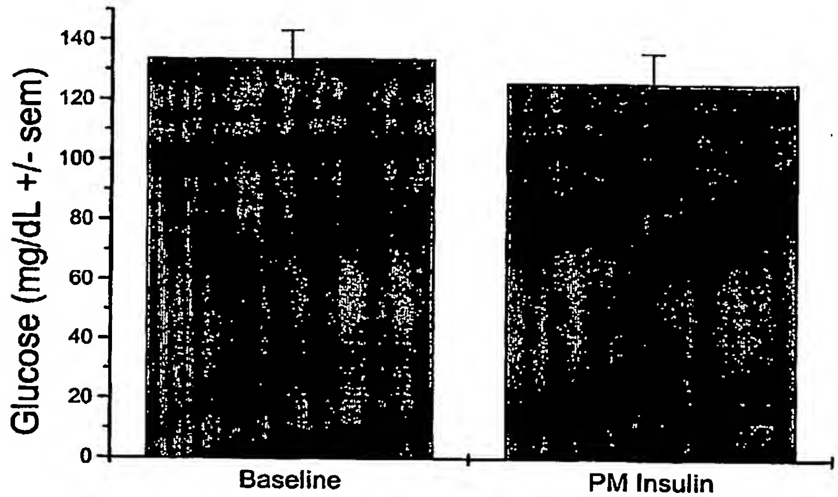


Figure 2

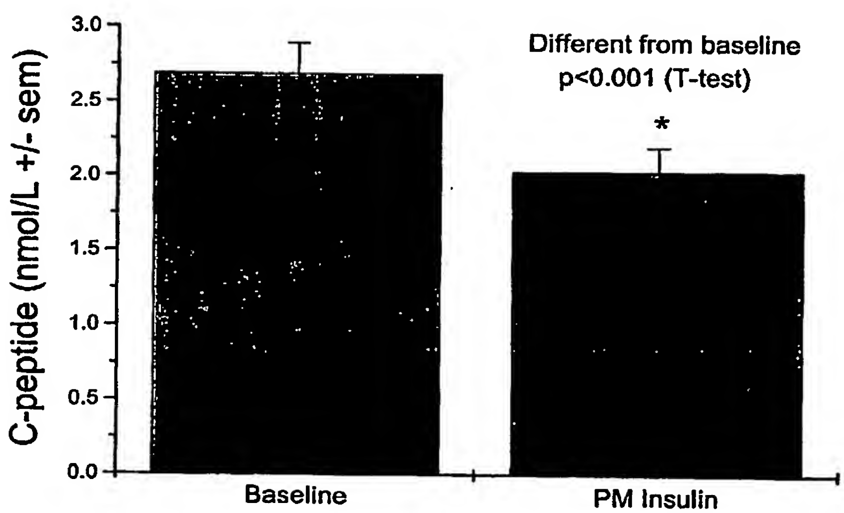


Figure 3

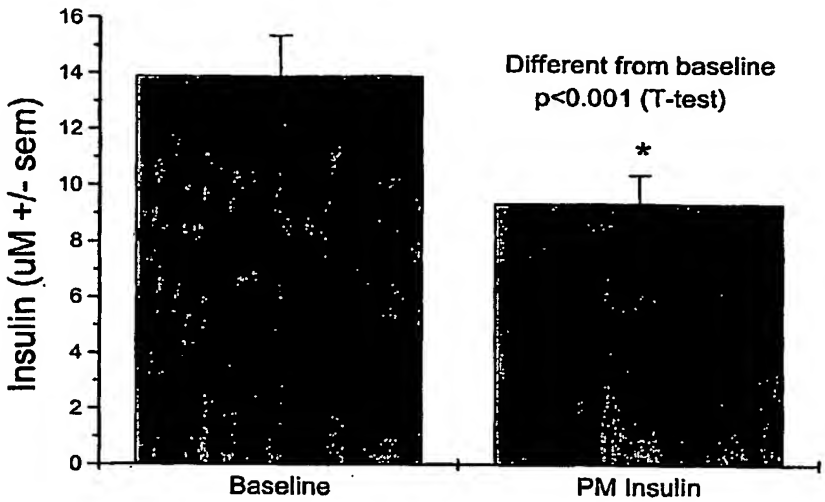

Different from baseline
p<0.001 (T-test)
*
Insulin (uM +/- sem)
16
14
12
10
8
6
4
2
0
Baseline
PM Insulin

Figure 4